Environmental Health Perspectives
Vol. 101(Suppl. 1): 3–281 (1993)

ABSTRACTS

TR-1 Guidelines for Carcinogenic Bioassay in Small Rodents

Note to the Reader: These guidelines were published to establish standard procedures for toxicology testing in rodents.

Report Date: February 1976

TR-2 Carcinogenesis Bioassay of Trichloroethylene (CAS No. 79-01-6)

Trichloroethylene (TCE), a halogenated chemical, has been tested for carcinogenicity in the National Cancer Institute's Carcinogenesis Bioassay Program. Trichloroethylene has been used primarily as a solvent in industrial degreasing operations. Other uses have been as a solvent in dry cleaning and food processing, as an ingredient in printing inks, paints. etc., and as a general anesthetic or analgesic.

Industrial grade (>99% pure) trichloroethylene was tested using 50 animals per group at 2 doses and with both sexes of Osborne-Mendel rats and $B6C3F_1$ mice. Twenty of each sex and species were maintained as matched controls, in addition to colony and positive carcinogen controls. Animals were exposed to the compound by oral gavage 5 times per week for 78 weeks. At the end of treatment, animals were observed until terminal sacrifice at 110 weeks for rats and 90 weeks for mice. A complete necropsy and microscopic evaluation of all animals (except 7 of the original 480) was conducted.

Two doses were used with animals started on test at approximately 6 weeks of age. The initial doses used in this test were the estimated maximum tolerated dose (MTD) and 1/2 MTD, as predicted from data obtained in a 6-week toxicity study. For rats, the initial doses were 1,300 and 650 mg/kg body weight. These were changed, based upon survival and body weight data, so that the "time-weighted average" doses were 549 and 1,097 mg/kg for both male and female rats. For mice, the initial doses were 1,000 and 2,000 mg/kg for males and 700 and 1,400 mg/kg for females. The doses were increased so that the "time-weighted average" doses were 1,169 and 2,339 mg/kg for male mice and 869 and 1,739 mg/kg for female mice.

Clinical signs of toxicity, including reduction in weight, were evident in treated rats. These, along with an increased mortality rate necessitated a reduction in doses during the test. In contrast, very little evidence of toxicity was seen in mice, so doses were increased slightly during the study. The increased mortality in treated male mice appears related to the presence of liver tumors.

A variety of neoplastic lesions were observed in rats with no significant difference between trichloroethylene-treated and control animals. The only lesion that might be attributed to the treatment was a chronic nephropathy found in both sexes and at both dose levels.

With both male and female mice, primary malignant tumors of the liver, i.e., hepatocellular carcinoma, were observed in high numbers. For males, 26/50 low dose and 31/48 high dose animals had hepatocellular carcinomas as compared with 1/20 matched controls and 5/77 colony controls. The differences between treated and matched control males at both doses were highly significant ($P<0.01$). For females, hepatocellular carcinomas were observed in 4/50 low dose and 11/47 high dose animals as compared with 0/20 matched controls and 1/80 colony controls. While the difference between the high dose female mice and matched controls was also highly significant ($P<0.01$), the difference at the low dose was less ($P=0.09$). For both male and female mice, age-adjusted tests for linear trend (dose response) were highly significant for hepatocellular carcinoma ($P<0.001$ for males and $P=0.002$ for females).

In male mice at the high doses, hepatocellular carcinomas were observed early in the study. The first was seen at 27 weeks; 9 others were found in male mice dying by the 78th week. The tumor was not observed so early in low dose male or female mice. The diagnosis of hepatocellular carcinoma was based on size, histologic appearance, and presence of metastasis, especially to the lung. No other lesion was significantly elevated ($P<0.05$) in treated mice. The incidence of hepatocellular carcinomas in the trichloroethylene-matched controls was typical of that observed in colony controls.

Carbon tetrachloride (CCl_4) was used as a positive control for the series of chlorinated chemicals which included trichloroethylene. While virtually all male and female mice developed hepatocellular carcinomas following carbon tetrachloride treatment, the response in the Osborne-Mendel rats was considerably less. Only about 5% developed hepatocellular carcinomas. Thus, there appears to be a marked difference in sensitivity to induction of carcinomas by chlorinated compounds between the $B6C3F_1$ mouse and the Osborne-Mendel rat.

The results of this carcinogenesis test of trichloroethylene clearly indicate that trichloroethylene induced a hepatocellular carcinoma response in mice.

While the absence of a similar effect in rats appears most likely attributable to a difference in sensitivity between the Osborne-Mendel rat and the B6C3F$_1$ mouse, the early mortality of rats due to toxicity must also be considered.

Synonyms: trichloroethene; acetylene trichloride; ethinyl trichloride; 1,1,2-trichloroethylene, TCE

Report Date: February 1976

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

Note: Trichloroethylene was subsequently studied by gavage in F344 rats and B6C3F$_1$ mice (See TR-243, reported in 1990) and also in four strains of rats (ACI, August, Marshall, and Osborne-Mendel) by gavage (See TR-273, reported in 1988).

TR-3 Bioassay of 1,1,1-Trichloroethane for Possible Carcinogenicity (CAS No. 71-55-6)

1,1,1-Trichloroethane is one of a group of halogenated hydrocarbons selected for testing in the Carcinogenesis Bioassay Program. The rationale for its selection included its structural relationship to carbon tetrachloride, its wide use in industry, its extensive exposure of humans, and the incomplete knowledge of its carcinogenic potential. In 1959, Browning reported that 1,1,2-trichloroethane was replacing the more toxic industrial solvents: trichloroethylene, tetrachloroethylene, and carbon tetrachloride. The Environmental Protection Agency permits 1,1,1-trichloroethane to be used as a solvent or cosolvent in pesticide formulations for the postharvest fumigation of citrus fruits.

The carcinogenesis bioassay of technical grade 1,1,1-trichloroethane was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. 1,1,1-Trichloroethane was administered orally by gavage in corn oil to 50 animals of each sex and species at two dose levels 5 days per week for 78 weeks.

Rats: The experiment was originally started using doses of 3,000 and 1,500 mg/kg of body weight. After a few weeks the study was terminated, and the animals discarded because of marked signs of intoxication. The experiment was restarted with rats 7 weeks of age that were put on doses of 1,500 and 750 mg/kg. There was a moderate depression of body weight in the first year of the study. During the second year a yellow discoloration of the fur of the lower abdomen and increased eye and nasal discharge and dyspnea were noted. Both males and females given the test chemical exhibited early mortality when compared with the untreated controls, and the statistical test for dose-related trend was significant ($P<0.04$). All surviving animals were killed at 117 weeks of age.

Mice: Male and female weanlings were started on test at 5 weeks of age and killed at 96 weeks of age. Initially, the doses for male and female mice were 4,000 and 2,000 mg/kg body weight. During the 10th week of the study, doses were increased to 5,000 and 2,500 mg/kg, since the animals apparently could tolerate a higher dose. Doses were again increased at week 20 to 6,000 and 3,000 mg/kg and maintained at these levels to the end of the study. Time-weighted average doses for the high- and low-dose mice were, respectively, 5,615 and 2,807 mg/kg. There was a moderate depression of body weight throughout the study in both sexes of mice, and the survival was significantly decreased. In the female mice, there was a positive dose-related trend ($P=0.002$) in the proportions surviving.

A variety of neoplasms were represented in both 1,1,1-trichloroethane-treated and matched-control rats and mice. However, each type of neoplasm has been encountered previously as a lesion in untreated rats or mice. The neoplasms observed are not believed attributable to 1,1,1-trichloroethane exposure, since no relationship was established between the dosage groups, the species, sex, type of neoplasm, or the site of occurrence. Even if such a relationship were inferred, it would be inappropriate to make an assessment of carcinogenicity of 1,1,1-trichloroethane on the basis of this test, because of the abbreviated life spans of both the rats and the mice.

Synonym: methylchloroform

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

TR-4 Bioassay of Dimethoate for Possible Carcinogenicity (CAS No. 60-51-5)

Dimethoate is the common name for the organophosphorous insecticide, o,o-dimethyl-S-(N-methylcarbamoyl-methyl)phosphorodithioate. This compound, which has been in use since 1956 as an insecticide and acaricide, is registered for insect and mite control on agricultural crops and ornamental plants. It is also registered as a residual fly spray in animal quarters.

A bioassay of the carcinogenicity of technical-grade dimethoate was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. The test material was administered in feed to groups of 50 rats of each sex at either of two concentrations for 80 weeks, followed by 35 weeks of observation. Initial doses were not well tolerated; therefore, they were reduced during the study. The "time-weighted average doses" for rats were 155 and 310 ppm for males and 192 and 384 ppm for females. All surviving rats were killed between 113 and 115 weeks.

Dimethoate was administered in feed to groups of 50 male and 50 female mice at two concentrations. Female mice received diets containing 200 and 500 ppm of dimethoate for 80 weeks; male mice received the same

dosage. However, high-dose males were returned to the control diet at 60 weeks, and low-dose males at 69 weeks. All surviving mice were killed between 93 and 94 weeks.

Tremors and hyperexcitability, both indications of dimethoate toxicity, were observed in the treated animals. However, it is considered that the low-dose group of rats and both dose groups of mice survived long enough to permit an evaluation of carcinogenicity. Pathologic evaluation revealed no statistically significant increase in tumors associated with dimethoate treatment in either species of animal, and it is concluded that there was no carcinogenic effect under the conditions of the experiment.

Synonyms: o,o-dimethyl-S-(N-methylcarbamoyl-methyl) phosphorodithioate

Report Date: January 1977

Levels of Evidence of Carcinogencity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-5 Bioassay of Proflavine for Possible Carcinogenicity (CAS No. 952-23-8)

Proflavine is a synthetic acridine dye which early in this century was found to have bacteriostatic and bacteriocidal properties when administered topically. During World War II it was widely used as a would antiseptic. With the advent of more specific and less toxic antibiotics, its clinical importance declined, until it was reintroduced recently in combination with ultraviolet light for the treatment of psoriasis and type-II herpesvirus infection.

A bioassay of the carcinogenicity of proflavine monohydrochloride hemihydrate was conducted using Fischer 344/CR rats and B6C3F$_1$ mice. The compound was administered in the diet at concentrations of 300 and 600 ppm to groups of 50 rats for 109 weeks and at concentrations of 200 and 400 ppm to groups of 50 mice for 104 weeks. The animals were subjected to necropsy and histopathologic evaluation as they died or at the end of their periods of treatment.

Average weights attained by high-dose groups were consistently lower than those of control groups; weights of low-dose groups showed essentially no differences from those of the controls. Survival rates of the treated rats and mice did not differ from those of the controls except for a lower rate among the female mice.

Five malignant neoplasms of the intestinal tract consisting of three leiomyosarcomas of the small intestine, a sarcoma near the colon area, and an adenocarcinoma of the small intestine were observed in five of the high-dose male rats. None were observed in other treatment or control groups. If these five intestinal neoplasms are considered together, they are significant at the $P = 0.026$ level using the Fisher exact test. A positive dose-related trend ($P = 0.034$) was also present for the three leiomyosarcomas.

The observed incidence of hepatocellular carcinoma in female mice was 4/50 (8%) in the control group, 20/49 (41%) in the low-dose group, and 22/50 (44%) in the high-dose group. The test for dose-related trend showed a level of significance of $P < 0.001$. In male mice, the observed incidence of hepatocellular carcinoma was 20/49 (41%) in the control group 28/49 (57%) in the low-dose group, and 30/50 (60%) in the high-dose group. The dose-related trend was significant at $P = 0.057$, and the high dose was significant at $P = 0.044$.

The unusually high incidence of hepatocellular carcinomas and hemangiosarcomas in control male mice and the unusually high incidence of malignant lymphomas in all groups of female mice in conjunction with the fact that a positive-control carcinogen was tested in the same room with these animals, raises a question of the validity of these bioassay results.

Synonym: 3,6-diaminoacridine

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-6 Bioassays of Nitrilotriacetic Acid (NTA) and Nitrilotriacetic Acid, Trisodium Salt, Monohydrate ($Na_3NTA \cdot H_2O$) for Possible Carcinogenicity (CAS No. 139-13-9) (NTA) (CAS No. 18662-53-8) ($Na_3NTA \cdot H_2O$)

Nitrilotriacetic acid (NTA) is a synthetic aminopolycarboxylic acid chelating agent used chiefly as a replacement for phosphates in detergents. NTA sequesters magnesium and calcium ions present in hard water, which would normally inhibit the activity of detergent surfactants. In December 1970, the detergent industry voluntarily suspended such applications of NTA in the United States following an unpublished government report indicating that the compound was teratogenic. During that year the annual production of NTA was 150 million pounds, of which 86-92% was used in detergents. Major nondetergent uses, for which NTA is still being produced, include water treatment, textile treatment, metal plating and cleaning, and pulp and paper processing. To a lesser extent, NTA is used in leather tanning, photographic development, synthetic rubber production, the manufacture of pharmaceuticals, agriculture (in herbicide formations and micronutrient solutions), and in the separation of rare-earth elements.

Bioassays for the carcinogenicity of nitrilotriacetic acid, trisodium salt, monohydrate ($Na_3NTA \cdot H_2O$) were conducted at Stanford Research Institute (SRI), using Fischer 344 rats and at Litton Bionetics, Inc. (LBI), using both Fischer 344 rats, and B6C3F$_1$ mice. Similar bioassays using rats and mice, were conducted at LBI on the

free acid, nitrilotriacetic acid (NTA). Each chemical was mixed in respective diets and administered *ad libitum*. The $Na_3NTA \cdot H_2O$ was tested in rats at SRI at 200, 2,000, and 20,000 ppm for a 24-month period. It was also tested in rats at LBI at 7,500 and 15,000 ppm and in mice at 2,500 and 5,000 ppm using 18-month feeding periods for both species. The NTA was tested in rats and mice at LBI at 7,500 and 15,000 ppm for the 18-month period. The numbers of animals used in tests at SRI were 24 of each sex for each dose group and for the controls; at LBI, 50 of each sex for each dose group and 20 of each sex for the controls. Since equimolar quantities of $Na_3NTA \cdot H_2O$ and NTA were not used, given concentrations of $Na_3NTA \cdot H_2O$ represented 30% less NTA than did equal concentrations of the free acid.

Average weights attained by high-dose groups of rats and mice were consistently lower than those of control groups. Less difference was observed with the low-dose groups. Survival, however, was not decreased by the compounds administered, except in rats given 20,000 ppm $Na_3NTA \cdot H_2O$.

Lesions of the urinary tract were found in most treated groups of both rats and mice. They were characterized, especially in the high-dose groups, by primary tumors of epithelial origin. These tumors were particularly significant since they were not found in the urinary tract of the control mice and only rarely occur spontaneously in the strains of animals on test. Lesions of the urinary tract were also characterized by hydronephrosis and/or nephritis in high-dose rats and by nephritis in both high- and low-dose mice.

Statistical evidence of the carcinogenicity of $Na_3NTA \cdot H_2O$ and NTA was provided by incidences of tumors at different sites in the urinary tract. For example, among animals given 20,000 ppm $Na_3NTA \cdot H_2O$ at SRI, tumors of the kidney occurred in male (treated, 9/24; untreated, 0/24; $P = 0.001$) and female (treated, 4/24; untreated, 0/24; $P = 0.054$) rats; tumors of the ureter in male (treated, 8/24; untreated, 0/24; $P = 0.002$) and female (treated, 6/24; untreated, 0/24; $P = 0.011$) rats; and tumors of the bladder, in female rats (treated, 5/24; untreated, 0/22; $P = 0.031$). Similarly, among animals given 15,000 ppm NTA at LBI, tumors of the bladder occurred in female rats (treated, 12/48; untreated, 0/18; $P = 0.014$) and tumors of the kidney occurred in male mice (treated, 24/44; untreated, 0/20; $P < 0.001$). Additional tests at LBI, using 15,000 and 7,500 ppm $Na_3NTA \cdot H_2O$ and 7,500 ppm NTA in male and female rats, 15,000 ppm NTA in female mice, and 7,500 ppm NTA in male mice, also induced tumors of the urinary tract, but in numbers too low to be statistically significant. Metastatic tumors, appearing to have arisen from primary tumors of the urinary tract, were found in 5/24 male and 5/24 female rats given 20,000 ppm $Na_3NTA \cdot H_2O$ at SRI and in one male rat given 15,000 ppm NTA at LBI; none were found in rats given lower doses or in mice.

Thus, NTA and $Na_3NTA \cdot H_2O$ were shown to be carcinogenic to the urinary tracts of both rats and mice at the higher doses tested. Lower doses, as delineated in this report, did not induce significant numbers of such lesions.

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

For Nitrilotriacetic Acid (NTA) at LBI:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

For Nitrilotriacetic Acid Trisodium Monohydrate at SRI:

Male Rats:	Positive
Female Rats:	Positive

For Nitrilotriacetic Acid Trisodium Monohydrate at LBI:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-7 Bioassay of Phenformin for Possible Carcinogenicity (CAS No. 114-86-3)*

Phenformin is a synthetic oral hypoglycemic agent used to control maturity-onset diabetes. Pharmacologically, phenformin acts to enhance anaerobic glycolysis, decrease gluconeogenesis, and inhibit intestinal absorption of glucose. This compound was selected for carcinogenicity testing since, in the treatment of diabetes, it is administered chronically.

A bioassay of the carcinogenicity of phenformin hydrochloride was conducted using Fischer 344 rats and $B6C3F_1$ mice. The compound was administered in the diet for 78 weeks to groups of 35 animals of each species and sex, using concentrations of 15,000 and 30,000 ppm for rats and concentrations of 1,200 and 2,500 ppm for mice. Treatment was followed by a period of observation of 26 weeks. Control groups consisted of 15 untreated animals of each species and sex.

Average weights attained by treated groups of rats and mice were consistently lower than those of control groups in all tests except that for male rats, in which case the weights shown by treated and control animals were indistinguishable. Survival was apparently unaffected in both species by treatment with phenformin, but was poor in mice due to intercurrent disease.

Tumors appearing in treated rats and mice were similar in type and number to those in controls, and no pathologic or statistical evidence of induction of tumors in these species by phenformin was found.

Synonym: 1-phenethylbiguanide hydrochloride

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

*The technical report states that Phenformin Hydrochloride (CAS No. 834-28-6) was the actual chemical tested rather than Phenformin in its pure form; therefore, the CAS number for Phenformin Hydrochloride is used to track this study in the NTP CHEMTRACK database.

TR-8 Bioassay of Chlordane for Possible Carcinogenicity (CAS No. 57-74-9)

Chlordane is a member of the cyclodiene group of chlorinated insecticides, which includes aldrin, dieldrin, endrin, heptachlor, and endosulfan. It was introduced in 1945 and was the first chlorinated cyclodiene developed for insect control. It is effective on a wide variety of insects of agricultural, industrial, and domestic importance. The compound was registered for use on more than 40 vegetable and 27 fruit crops. About a third of the amount used in the United States is applied to pests of the home, garden, lawn, and turf.

A bioassay of analytical-grade chlordane for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and B6C3F$_1$ mice. Groups of 50 rats of each sex were administered low or high concentrations of the chlordane for 80 weeks, then observed for 29 weeks. Because of toxic effects, doses were reduced for both male and female rats during the course of the tests. Time-weighted average doses used for the male rats were 203.5 and 407.0 ppm; for the females, 120.8 and 241.5 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male and 50 untreated female rats from similar bioassays of five other compounds. All surviving rats were killed at 109 weeks.

Groups of 50 mice of each sex were administered the test material at low or high concentrations for 80 weeks, then observed for 10 weeks. The low- and high-dose groups were tested at different calendar times, but each of the treated groups was tested along with a concurrent control. Because of toxic effects, doses were reduced for female mice during the course of the tests; however, it was possible to increase the doses for the male mice. The time-weighted average doses used for the male mice were 29.9 and 56.2 ppm; for the females, 30.1 and 63.8 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 70 untreated male and 80 untreated female mice from similar bioassays of five other compounds. All surviving mice were killed at 90-91 weeks.

The effects of chlordane on body weights and other clinical signs in rats and mice indicated that the dosages used were near the maximum permissible. This was evident in that the average body weights of the high-dose male and female rats were consistently lower than those of the untreated controls, while differences between the low-dose and control rats were negligible. Body weights of mice given either low or high doses showed little or no effect of the chlordane; however, other adverse clinical signs were seen with greater frequency than in control mice.

The effects of chlordane on survival rates indicated that mortality was dose-related for female rats and for male mice. However, a substantial proportion of most groups of animals survived to an age at which tumors could be expected to appear; male control rats, for unknown reasons, showed an abnormally low survival rate.

Hepatocellular carcinoma showed a highly significant dose-related trend for mice, using either matched controls (for males, controls 2/18, low dose 16/48, high dose 43/49, $P<0.0001$; for females, controls 0/19, low dose 3/47, high dose 34/49, $P<0.0001$) or pooled controls (for males, controls 17/92, $P<0.0001$; for females, controls 3/78, $P<0.0001$). These high levels of significance were maintained when hepatocellular carcinoma was combined with nodular hyperplasia or when the data were subjected to life-table adjustment. No other tumors were found in mice in sufficient numbers to justify analysis.

In contrast to findings with mice, hepatocellular carcinoma failed to appear at a significant rate of incidence in rats administered chlordane. Further, the number of lesions of the liver in rats did not become significant with the addition of nodular neoplasia or with the application of life-table adjustment to the data.

There was significant statistical evidence for the induction in treated male rats of proliferative lesions of follicular cells of the thyroid and of malignant fibrous histiocytoma, but these findings were discounted because the rates of incidence were comparatively low and/or are known to be variable in control rat populations.

It is concluded that under the conditions of this bioassay chlordane is carcinogenic for the liver in mice.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-9 Bioassay of Heptachlor for Possible Carcinogenicity (CAS No. 76-44-8)

Heptachlor is a member of the cyclodiene group of chlorinated insecticides (aldrin, dieldrin, endrin, chlordane, heptachlor, and endosulfan) that was developed in the 15 years following World War II. It was registered as a commercial pesticide in 1952 for foliar, soil, and structure applications and for malarial control programs; after 1960 it was used primarily in soil applications against agricultural pests and to a lesser extent against termites.

A bioassay of technical-grade heptachlor for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and B6C3F$_1$ mice. Groups of 50 rats of each sex were administered low or high concentrations of the heptachlor for 80 weeks, then observed for 30 weeks. Doses for females were first increased, but because of toxic effects the doses were then reduced twice for both male and female rats during the

remaining course of the tests. Time-weighted average doses used for the male rats were 38.9 and 77.9 ppm; for the females, 25.7 and 51.3 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male and 50 untreated female rats from similar bioassays of five other compounds. All surviving rats were killed at 110-111 weeks.

Groups of 50 mice of each sex were administered the test material at low or high concentrations for 80 weeks, then observed for 10 weeks. The low- and high-dose groups were tested at different calendar times, but a concurrent control group was started with each. Because of toxic effects, doses were reduced once for the males at 17-18 weeks after the initiation of tests; twice for the females, at 17 and 30 weeks, after the initiation of tests. The time-weighted average doses used for the male mice were 6.1 and 13.8 ppm; for the females, 9 and 18 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 90 untreated male and 70 untreated female mice from similar bioassays of five other compounds. All surviving mice were killed at 90-91 weeks.

The effects of heptachlor on body weights and other clinical signs in rats and mice indicated that the dosages used were near the maximum permissible. This was evident in that average body weights of rats treated with high doses were consistently lower than those of untreated controls, while body weights of low-dose rats were unaffected. Body weights of mice given either high or low doses showed little or no differences from those of control mice; however, other adverse clinical signs were found in high-dose mice, predominantly in the females.

The effects of heptachlor on survival rates indicated that mortality was dose-related for both female rats and female mice, but not for males of either species. However, a substantial proportion of all groups of animals survived to an age at which tumors could be expected to appear.

In mice, hepatocellular carcinoma showed a highly significant dose-related trend in both males (matched controls 5/19, low dose 11/46, high dose 34/47, $P = 0.001$) and females (control 2/10, low dose 3/47, high dose 30/42, $P < 0.0001$). When pooled controls were used for the comparison, the significance of the trend in males increased to $P < 0.0001$. Comparably high levels of significance were attained when the data were subjected to life-table adjustment. No other tumors were found in mice in sufficient numbers to justify analysis.

In marked contrast to the findings observed in mice, no hepatic tumors were observed in rats administered heptachlor. There was significant statistical evidence for the induction of proliferative lesions of follicular cells of the thyroid in treated female rats, but this finding was discounted because the rates of incidence were comparatively low and are known to be variable in control rat populations.

It is concluded that under the conditions of this bioassay, heptachlor is carcinogenic for the liver in mice.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Positive

TR-10 Bioassay of Dichlorvos for Possible Carcinogenicity (CAS No. 62-73-7)

Dichlorvos is an organophosphate insecticide with contact and vapor action. It has been used widely for control of agricultural, industrial, and domestic pests since the 1950's. Dichlorvos is available in oil solutions, emulsifiable concentrations, and aerosol formulations; the impregnation of dichlorvos in a polyvinyl chloride base (pellets, strips, blocks, etc.) for delayed release is a widely used method for the control of pests in domestic and industrial situations.

A bioassay for the possible carcinogenicity of technical-grade dichlorvos was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. The test material was administered in the diet at two concentrations for 80 weeks to groups of 50 animals of each species and sex. The test animals were held for observation, and surviving rats were killed at 110-111 weeks and surviving mice at 92-94 weeks from initiation of the study. Initial doses in both species were not well tolerated and they were lowered after a few weeks. Time-weighted average doses for both males and females were 150 and 326 ppm for rats and 318 and 635 ppm for mice. The matched controls consisted of 10 rats of each sex and 10 mice of each sex; the pooled controls consisted of 60 rats of each sex, 100 male mice, and 80 female mice. All surviving rats were killed at 106 to 109 weeks; surviving mice, at 92 to 94 weeks.

After the doses were reduced, no toxic signs directly attributable to the compound were observed. However, average weights of high-dose animals were slightly depressed. Survival was not dose-related in either species. Microscopic study of the tissues of treated animals and matched and pooled controls revealed no statistically significant increase in the incidence of tumors attributable to exposure to dichlorvos in either animal species. The significance of the three esophageal tumors in male and female mice and of malignant fibrous histiocytomas in male mice is unclear and there is insufficient evidence to indicate they were associated with dichlorvos treatment. Thus under the conditions of this study, dichlorvos was not demonstrated to be carcinogenic.

Synonym: 2,2-dichlorovinyl dimethylphosphate

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

Note: Dichlorvos was subsequently studied by gavage in F344 rats and $B6C3F_1$ mice (See TR-342, reported 1989).

TR-11 Bioassay of Trisodium Ethylenediaminetetraacetate Trihydrate (EDTA) for Possible Carcinogenicity (CAS No. 150-38-9)

A bioassay of the chelating agent, trisodium ethylenediaminetetraacetate trihydrate ($Na_3EDTA{\cdot}3H_2O$), for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and $B6C3F_1$ mice. The chemical was administered to 50 males and 50 females of each species at low and high concentrations, 3,750 and 7,500 ppm, for 103 weeks. Matched-control groups were composed of 20 males and 20 females of each species.

No compound-related signs of clinical toxicity were noted. Although a variety of tumors occurred among test and control animals of both species, no tumors were related to treatment. Since survival was satisfactory and showed no consistent variation among test and control groups, the absence of treatment-related tumors could not be attributed to early mortality.

Synonym: EDTA

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-12 Bioassay of Endrin for Possible Carcinogenicity (CAS No. 72-20-8)

Endrin is an organochlorine pesticide having a structural characteristic of the cyclodiene group, which includes aldrin (CAS No. 309-00-2), dieldrin (CAS No. 60-57-1), chlordane (CAS No. 57-74-9), heptachlor (CAS No. 76-44-8), and endosulfan (CAS No. 115-29-7). It is the most acutely toxic compound in the cyclodiene group but is less persistent in the environment than DDT or dieldrin. As an insecticide, it is currently used for small grains, sugarcane, and cotton; as an avicide, for forest seed and perch applications; and as a rodenticide, for forest seed and orchard soil applications.

A bioassay of technical-grade endrin for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice. Groups of 50 rats of each sex were administered one of two doses of endrin for 80 weeks, then observed for 31 or 34 weeks. The doses used for the male rats were 2.5 or 5 ppm. The initial doses of 5 or 10 ppm used for the females were not well tolerated and were reduced during the study. The time-weighted average doses used for the females were 3 or 6 ppm. Matched controls consisted of groups of 10 rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 40 untreated male and 40 untreated female rats from similar bioassays of other test chemicals. All surviving rats were killed at 110 to 114 weeks.

Groups of 50 mice of each sex were administered endrin at one of two doses for 80 weeks, then observed for 10 or 11 weeks. Initial doses of 2.5 or 5 ppm used for the males were not well tolerated and were reduced during the study. The time-weighted average doses used for the males were 1.6 or 3.2 ppm; the doses used for the females were 2.5 or 5 ppm. Matched controls consisted of groups of 10 mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 50 untreated male and 50 untreated female mice from similar bioassays of other test chemicals. All surviving mice were killed at 90 or 91 weeks.

The clinical signs observed in both rats and mice indicated that the doses of endrin used were near the maximum tolerated doses. In mice these signs included hyperexcitability, a manifestation of toxicity of the organochlorine pesticides. However, mean body weights of the rats and mice were not affected by administration of endrin.

Although the survival of the high-dose male mice at the end of the study was markedly lower than that of the controls, the survivals of the low- and high-dose female mice and male and female rats were unaffected by the endrin. The survival of the low-dose male mice could not be evaluated, due to the accidental administration of excessive quantities of endrin to this group during week 66. However, a substantial portion of all groups of rats and mice survived to an age at which tumors could be expected to occur.

In rats, the combination of adenomas and carcinomas of the adrenal occurred at the following incidences – males: pooled controls 2/44, matched controls 2/9, low-dose 4/46, high-dose 8/44; females: pooled controls 4/46, matched controls 3/9, low-dose 16/49, high-dose 7/47. These incidences did not show consistent statistical significance. Furthermore, the incidences of the tumors in the matched controls of either sex were higher than those of the corresponding pooled controls, and the incidences in the matched controls equaled or exceeded those in any of the respective dosed groups. Thus, these tumors cannot be clearly related to administration of the test chemical.

In mice, no tumors occurred in dosed groups at incidences that were significantly higher than those in pooled or matched controls.

It is concluded that under the conditions of this bioassay, endrin was not carcinogenic for Osborne-Mendel rats or for $B6C3F_1$ mice.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-13 Bioassay of Tetrachloroethylene for Possible Carcinogenicity (CAS No. 127-18-4)

Tetrachloroethylene is one of a group of halogenated organic solvents selected by the National Cancer Institute (NCI) for inclusion in the Carcinogenesis Bioassay Program. These solvents were selected on the basis of large-scale production, extensive use, and lack of adequate chronic toxicity data.

The bioassay of U.S.P.-grade tetrachloroethylene for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Tetrachloroethylene in corn oil was administered by gavage at either of two dosages to groups of 50 male and 50 female animals of each species, 5 days a week, over a period of 78 weeks followed by an observation period of 32 weeks for rats and 12 weeks for mice.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The high and low time-weighted average dosages of tetrachloroethylene in the chronic study were 941 and 471 mg/kg/day for the male rats, 949 and 474 mg/kg/day for the female rats, 1,072 and 536 mg/kg/day for the male mice, and 772 and 386 mg/kg/day for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same time that dosed animals were gavaged with tetrachloroethylene mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals received no gavage treatments.

No significant increased incidence of neoplastic lesions was observed in treated rats. In both dosed and control rats, respiratory disease was observed with increasing frequency for the latter part of the first year until termination of the bioassay. Lesions indicative of pneumonia were observed in nearly all rats at necropsy. A high incidence of toxic nephropathy was observed in treated rats. Toxic nephropathy was noted in rats that died early in the study (as early as week 20 for male rats and week 28 for female rats). Mortality of rats was dose-related. Fifty percent of the high dose males had died by week 44 and 50 percent of the high dose females had died by week 66.

In both male and female mice, administration of tetrachloroethylene was associated with a significantly increased incidence of hepatocellular carcinoma. Hepatocellular carcinomas were observed in 2/17 (12 percent) untreated control males, 2/20 (10 percent) vehicle control males, 32/49 (65 percent) low dose males, 27/48 (56 percent) high dose males, 2/20 (10 percent) untreated control females, 0/20 vehicle control females, 19/48 (40 percent) low dose females, and 19/48 (40 percent) high dose females. Hepatocellular carcinomas metastasized to the kidney in one untreated control male and to the lung in three low dose males, one low dose female, and one high dose female. Toxic nephropathy, similar to that observed in rats, was also observed in treated but not control mice.

Fisher exact tests indicated a highly significant increased incidence of hepatocellular carcinoma for each dosed group compared to each control group. Cochran-Armitage tests showed a highly significant positive association between increased dosage and elevated tumor incidence. Time-adjusted analyses, based on Kaplan and Meier survival curves, indicated that the estimated probability of observing hepatocellular carcinoma by week 91 was 1.00 in a dosed male mouse and 0.938 in a dosed female mouse.

The results of the bioassay of tetrachloroethylene in Osborne-Mendel rats do not allow an evaluation of the carcinogenicity of this compound due to the high rate of early death among the treated animals. However, under the condition of this study, tetrachloroethylene is a liver carcinogen in $B6C3F_1$ mice of both sexes.

Synonyms: perchloroethylene; carbon dichloride

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Positive
Female Mice:	Positive

Note: Tetrachloroethylene was subsequently studied by inhalation in F344 rats and $B6C3F_1$ mice (See TR-311, reported 1986).

TR-14 Bioassay of Lindane for Possible Carcinogenicity (CAS No. 58-89-9)

Lindane is an organochlorine pesticide that is registered for use in soil, foliar, and seed treatment for a large variety of fruit and vegetable crops, and for use on livestock, pets, and agricultural premises. Residues of lindane may be persistent in soil and foods. There may also be direct human exposure to lindane through its use in pharmaceutical preparation or in public health pest control.

A bioassay for possible carcinogenicity of lindane was conducted by administering the test chemical in the diet to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered lindane at one of two doses for 80 weeks, then observed for 29-30 weeks. Time-weighted average doses for males were 236 or 472 ppm; those for females were 135 or 270 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 45 untreated male and 45 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-110 weeks.

Groups of 50 mice of each sex were administered lindane at one of two doses, either 80 or 160 ppm, for 80 weeks, then observed for an additional 10-11 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 40 untreated

male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-91 weeks.

Neither the mean body weights of rats nor those of mice showed consistent effects from the administration of lindane. The physical condition of the surviving treated mice deteriorated during the final 6 weeks on study. Except for the female matched-control group of rats, survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors.

In rats, no tumors occurred at a statistically significant incidence in the treated groups of either sex.

In mice, the incidence of hepatocellular carcinoma in low-dose males was significant when compared with that in the pooled controls (controls 5/49, low-dose 19/49, P = 0.001). This finding, by itself, is insufficient to establish the carcinogenicity of lindane. The incidence of hepatocellular carcinoma in high-dose male mice (9/46) was not significantly different from that in the matched (2/10) or pooled controls.

It is concluded that under the conditions of this bioassay, lindane was not carcinogenic for Osborne-Mendel rats or $B6C3F_1$ mice.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Netative

TR-15 Bioassay of Captan for Possible Carcinogenicity (CAS No. 133-06-2)

Captan is a broad-spectrum fungicide which inhibits mycelial growth from germinating fungus spores. As a result, it has effective protection action, although it will not eradicate a preexisting infection. Because captan is a nonpersistent fungicide, directions for use indicate that it should be reapplied every week as necessary to maintain control. It has been one of the most widely used fungicides since its introduction in 1950.

A bioassay of technical-grade captan for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered one of two doses of captan for 80 weeks, then observed for 33 or 34 weeks. The time-weighted average doses for both sexes of rats were 2,525 or 6,050 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 75 untreated male and 75 untreated female rats from similar bioassays of six other test chemicals. All surviving rats were killed at 113-114 weeks.

Groups of 50 mice of each sex were administered the test material at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 11 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 80 untreated male and 80 untreated female mice from similar bioassays of six other test chemicals. All surviving mice were killed at 90-91 weeks.

The mean body weights of both low- and high-dose rats and high-dose mice were lower than those of the matched controls throughout most of the study. Mortality rates did not show statistically significant dose-related trends in either sex of either species.

In rats, a positive dose-related trend and a difference between incidences of tumors in high-dose and pooled-control groups were found in females when the data for adrenal cortical adenoma were combined with those for adrenal cortical carcinoma (pooled controls, 0/64, low-dose 2/50, high-dose 3/47, P = 0.047). There was also a positive dose-related trend for the incidence of C-cell adenoma of the thyroid in female rats (pooled controls 1/66, low-dose 1/49, high-dose 4/44, P = 0.035). These endocrine tumors in female rats are believed to have been spontaneous, and not related to treatment.

In mice, the incidences of polypoid carcinoma (adenocarcinoma in adenomatous polyp) of the duodenum were statistically significant using tests for a positive dose-related trend both in male mice (pooled controls 0/68, low-dose 1/43, high-dose 3/46, P = 0.033) and in female mice (pooled controls 0/68, low-dose 0/49, high-dose 3/48, P = 0.022). When the incidences of adenomatous polyp, NOS (not otherwise specified), were combined with those of polypoid carcinoma for statistical analysis, the tests for male mice indicated a substantial increase in significance (pooled controls 0/68, low-dose 3/43, high-dose 5/46, P = 0.008).

It is concluded that under the conditions of this bioassay, tumors in the duodenum of $B6C3F_1$ mice were associated with treatment with captan, but there was no convincing evidence that the tumors observed in Osborne-Mendel rats were related to treatment.

Synonym: N-((trichloromethyl)thio)-4-cyclohexene-1,2-dicarboximide

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-16 Bioassay of Phosphamidon for Possible Carcinogenicity (CAS No. 13171-21-6)

Phosphamidon is an organophosphorus compound used as a broad-spectrum insecticide in agriculture since 1956. It is toxic both systemically and by contact, and acts through the inhibition of cholinesterase. Phosphamidon is currently registered for use by both ground and aerial

applications on vegetables, fruits and field crops with tolerances for residues from 0.1 to 1.0 ppm.

A bioassay of technical-grade phosphamidon for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. The test material was administered in feed to 50 rats and 50 mice of each sex at one of two doses, either 80 or 160 ppm. The rats were fed the test chemical for 80 weeks, then observed without compound administration for 30 or 31 weeks; the low-dose male mice were fed for 71 weeks, then observed for 19 weeks; the high-dose male mice were fed for 62 weeks, then observed for 28 weeks; and the low- and high-dose female mice were fed for 80 weeks, then observed for 10 or 11 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 85 male and 85 female untreated rats or 80 male and 80 female untreated mice from similar bioassays of eight other test chemicals. All surviving rats were killed at 110 or 111 weeks; all surviving mice were killed at 90 or 91 weeks.

Hyperexcitability and tremors, both indications of phosphamidon toxicity, were observed in dosed rats and mice. However, sufficient numbers of all groups of both species were at risk for the development of late-appearing tumors.

In male rats, the combined incidence of hemangiomas and hemangiosarcomas in the spleen showed a statistically significant ($P = 0.012$) dose-related trend. However, the comparison with matched controls was not significant, and the historical records of this laboratory on untreated males of this strain show a tumor incidence of 6/240 (3%) with incidences in individual control groups as high as 3/9 (33%) and 2/9 (22%), compared with 5/49 (10%) seen in the high-dose group in this study. No hemangiomas or hemangiosarcomas were found in the females.

In female rats, the Cochran-Armitage test for dose-related trend was significant ($P = 0.003$) for C-cell adenomas and carcinomas of the thyroid when pooled controls were compared with the dosed groups. The incidences of these tumors were also significant when low-dose females ($P = 0.003$) and high-dose females ($P = 0.004$) were compared directly with pooled controls. However, the historical records of this laboratory show a tumor incidence of 16/235 (7%) in untreated female rats of this strain of female rats, with incidences in individual control groups as high as 3/9 (33%) and 3/10 (30%); these data are therefore considered marginal and insufficient to establish an association between the tumors and administration of the chemical. In males, the incidence of these tumors was not statistically significant.

In mice, no tumor occurred at a higher incidence in dosed animals than in controls.

It is concluded that under the conditions of this bioassay, technical-grade phosphamidon was not carcinogenic for $B6C3F_1$ mice. The data obtained in this bioassay with Osborne-Mendel rats are insufficient to allow the interpretation that technical-grade phosphamidon is carcinogenic in this species.

Synonym: dimethyl 2-chloro-2-diethyl-carbamoyl-1-methylvinyl phosphate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-17 Bioassay of Photodieldrin for Possible Carcinogenicity (CAS No. 13366-73-9)

Photodieldrin is a photochemical conversion product of dieldrin. Although it has never been produced commercially, photodieldrin was selected for testing in 1969 because it was a photochemical conversion product of dieldrin. At that time dieldrin was used extensively as a pesticide.

A bioassay of dieldrin-free photodieldrin (synthesized by Gulf South Research Institute) for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were initially administered photodieldrin at one of two doses, either 5 or 10 ppm. Because of neurotoxic signs, doses in the females were reduced after 30 weeks. Total periods of treatment for low- and high-doge males and low-dose females were 80 weeks, followed by periods of 31 or 32 weeks of additional observation; the total period of treatment for the high-dose females was 59 weeks, followed by a period of additional observation of 53 weeks. The time-weighted average doses for the females were 3.4 or 7.5 ppm. Matched controls consisted of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 65 untreated male and 65 untreated female rats from similarly performed bioassays of six other test chemicals. All surviving rats were killed at 111-112 weeks.

Groups of 50 mice of each sex were administered photodieldrin at one of two doses, either 0.32 or 0.64 ppm, for 80 weeks, then observed for an additional 13 weeks. Matched controls consisted of groups of 10 untreated mice of each sex at each dose; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 60 untreated male and 60 untreated female mice from similarly performed bioassays of six other test chemicals. All surviving mice were killed at 93 weeks.

Mean body weights attained by low- and high dose male and female rats and mice were essentially unaffected by photodieldrin. Convulsions and hyperactivity were noted in treated male and female rats and in male mice. Mortality rates of either sex or either species were not affected by treatment.

In rats, benign tumors (adenoma and fibroadenoma) of the mammary gland in females showed a dose-related trend ($P = 0.039$) compared with matched, but not pooled,

controls (8/72 pooled controls, 0/9 matched controls, 5/50 low-dose, 10/49 high-dose). Adenocarcinoma of the mammary gland occurred in two additional low-dose females. The incidences of these tumors in either of the treated groups were not significantly higher than those in the control groups using either matched or pooled controls. Three papillary and follicular-cell adenomas and one papillary adenocarcinoma of the thyroid occurred in the low-dose females, giving a statistically significant increase over the pooled controls (P = 0.022), but these thyroid tumors did not occur in the high-dose animals. The dose-related trend was not statistically significant using either pooled or matched controls, and the incidence in the low-dose group is not greater than that in the historical controls. In male rats, the incidence of hemangiomas showed a statistically significant dose-related trend (P = 0.021) using pooled controls, but the direct comparison of the three hemangiomas in the high-dose group with the pooled-control group was not statistically significant. Furthermore, three hemangiomas is a small number, and the tumors occurred in more than one anatomic site (two in the spleen, one in subcutaneous tissue). The occurrence of these tumors cannot clearly be associated with treatment.

In mice, there were no tumors that were statistically significant in treated groups of either sex.

It is concluded that under the conditions of this bioassay, photodieldrin was not carcinogenic for Osborne-Mendel rats or B6C3F$_1$ mice.

Synonym: 1,1,2,3,3a,7a-hexachloro-exo-5,6-epoxydecahydro-2,4,7-metheno-1H-cyclopenta[a]pentalene

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-18 Bioassay of 3,3'-Iminobis-1-propanol Dimethanesulfonate (Ester) Hydrochloride (IPD) for Possible Carcinogenicity (CAS No. 3458-22-8)

3,3'-Iminobis-1-propanol dimethanesulfonate (ester) hydrochloride, hereinafter called IPD, was synthesized from bis(3-hydroxypropyl)amine and methanesulfonic acid anhydride. It was found to have antitumor activity against a number of experimental tumors that were naturally resistant to nitrogen mustard and has been used in Japan for the treatment of myelogenous leukemia. IPD was selected for carcinogen bioassay as one agent in a series of anticancer drugs that are administered chronically in the treatment of human cancer.

A bioassay of IPD for possible carcinogenicity was conducted by administering the test chemical intraperitoneally to Sprague-Dawley rats and B6C3F$_1$ mice.

The IPD was injected three times per week to groups of 35 animals, using doses of 12, 24, or 48 mg/kg for the rats, and 20 or 40 mg/kg for the mice. Rats at 12 mg/kg were treated for 52 weeks. Because of the toxicity of the chemical, administration of IPD for the group receiving 24 mg/kg was discontinued at week 34. Rats receiving 48 mg/kg were treated until all had died at week 23 (males) and week 27 (females). Both groups of mice were treated for 52 weeks. All survivors were killed after post-administration periods that varied among groups.

With rats, untreated and vehicle-control groups, each consisting of 10 males and 10 females, were started with the high- and mid-dose groups and additional untreated and vehicle-control groups of the same size were started with the low-dose groups. With mice, untreated and vehicle-control groups each consisted of 15 males and 15 females.

The toxicity of IPD was associated with lower mean body weights and lower rates of survival of both the rats and mice. The shortened life spans, particularly in the rats, reduced the likelihood of the development of tumors.

In rats, peritonitis and fibrous adhesions, possibly, from direct irritation by the test chemical were observed in most treated rats at necropsy. Sarcoma, fibroma, or fibrosarcoma of the peritoneum occurred in two low-dose male, one mid-dose male, and one mid-dose female rats, but not in any control animals. Because of this low incidence, and because irritation by the test chemical have been involved in the pathogenesis, these tumors may have been due to local effects of the chemical.

In mice, lymphomas were observed at the following incidences (males: controls 0/14, low-dose 0/26, high-dose 3/21; females: controls 1/15, low-dose 2/29, high-dose, 6/27). The Tarone test for life-table analysis of the probability of survival without lymphoma indicated a significant positive dose-related increase of lymphomas with a probability level of 0.011 for male mice and 0.003 for female mice.

Squamous-cell carcinoma was noted in the mice (low-dose males 6/26, high-dose females 2/27). Seven of these tumors were observed in subcutaneous tissue in the inguinal region near the sites of injection. Although not statistically significant, this tumor may be associated with administration of IPD.

Tumors of the peritoneum in rats and tumors in the subcutaneous tissue in mice may have been due to local effects related to administration of the test chemical. The lymphomas in mice, although marginally significant, were too few in number to clearly be related to dosing.

Conclusions from this study are limited by early deaths and toxicity, but the appearance of tumors in the peritoneum near the injection sites in both rats and mice indicate the carcinogenic potential of IPD.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-19 Bioassay of Procarbazine for Possible Carcinogenicity (CAS No. 366-70-1)

Procarbazine is a methylhydrazine derivative which has been shown to have effective antineoplastic activity in advanced Hodgkin's disease and in oat-cell carcinoma of the lung. It has also been shown to have carcinogenic activity in rats and mice.

A bioassay of procarbazine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 34 or 35 males and 35 or 36 females of both species were administered procarbazine at one of two doses, either 15 or 30 mg/kg for rats, and either 6 or 12 mg/kg for mice. Injections were made three times per week for 26 weeks for the rats and 52 weeks for the mice. Following the periods of injection, the dosed animals were observed for a maximum period of 60 weeks for rats and 33 weeks for mice, depending on survival. Vehicle controls, used for statistical evaluation, consisted of 10 rats and 15 mice of each sex, administered saline solution on the same schedule as the test solution; the same numbers of rats and mice served as untreated controls. Pooled controls consisted of the vehicle controls from this bioassay together with the vehicle controls from two other bioassays similarly performed at the same laboratory. The pooled-control groups consisted of 40 rats of each sex and 45 mice of each sex. Surviving rats were killed at 86 weeks and surviving mice were killed at 85 weeks.

Mean body weights of low- and high-dose rats and of high- dose female mice were lower than those of the vehicle controls. Survival rates of both rats and mice showed significant dose-related trends.

In rats, malignant lymphomas, adenocarcinomas of the mammary gland, and the combination of olfactory neuroblastomas, adenocarcinomas, or carcinomas of the brain, olfactory bulb, or cerebrum were induced in statistically significant numbers.

In mice, malignant lymphomas or leukemias, olfactory neuroblastomas or undifferentiated carcinomas, alveolar/bronchiolar adenomas, and adenocarcinomas of the uterus were induced in statistically significant numbers.

It is concluded that under the conditions of this bioassay, procarbazine was carcinogenic for both Sprague-Dawley rats and $B6C3F_1$ mice, producing several types of tumors in both of these two species.

Synonym: N-iso-propyl-α-(2-methylhydrazino)-p-toluamide hydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-20 Bioassay of Dapsone for Possible Carcinogenicity (CAS No. 80-08-0)

Dapsone is the parent chemical of the sulfone drugs, and the major therapeutic agent in this group for the treatment of leprosy. It is also administered to treat dermatitis herpetiformis and malaria, and is used in combination with radiotherapy in the treatment of gynecologic neoplasms. Dapsone is also sold for use as an accelerator in epoxy resins.

A bioassay of dapsone for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered dapsone at one of two doses, either 600 or 1,200 ppm for rats and either 500 or 1,000 ppm for mice. The rats and mice were treated for 78 weeks; the rats were then observed for 26-28 weeks, the mice for 28-30 weeks. Matched controls consisted of groups of 15 untreated rats and 14 untreated mice of each sex, pooled controls, used for statistical evaluation, consisted of the matched controls combined with 30 male and 30 female untreated rats and 29 male and 29 female untreated mice from similarly performed bioassays of two other test chemicals. All surviving rats were killed at 104-106 weeks, all surviving mice at 106-108 weeks.

Treated rats and mice had lower mean body weights than the corresponding controls; when treatment was discontinued at week 78, both species showed some increase in body weight. Survival among rats was unaffected by treatment with dapsone; adequate numbers of animals survived for meaningful statistical analyses of the incidences of tumors. Dapsone did not adversely affect the survival of mice, as shown by the test for positive dose-related trend. Suppurative bronchopneumonia was found in some mice in all matched-control and treated groups. Several control males died early in the study, while survival of the other groups of mice was not affected until week 75.

Among rats, mesenchymal tumors of the abdominal organs or peritoneal tissues occurred in 13/35 low-dose males and 22/33 high-dose males. None occurred among control males or among control or treated females. The most commonly occurring tumors were fibroma, fibrosarcoma, or sarcoma, NOS (not otherwise specified), of the spleen and the peritoneum. In male rats, these mesenchymal tumors of the spleen occurred in a statistically significant incidence in both treated groups (low-dose 6/34, $P = 0.006$; high-dose 14/32, $P < 0.001$) when compared with pooled controls. In the peritoneum, the incidences of these mesenchymal tumors were significant in both treated groups (low-dose 5/35, $P = 0.014$; high-dose 6/33, $P = 0.005$) when compared with the pooled controls. No tumors related to treatment were found in female rats.

Among the mice, there were no tumors that could clearly be related to treatment.

It is concluded that under the conditions of this bioassay, dapsone was not carcinogenic for female Fischer 344 rats or $B6C3F_1$ mice of either sex. Dapsone was carcinogenic

(sarcomagenic) for male Fischer 344 rats, causing mesenchymal tumors in the spleen and the peritoneum.

Synonym: 4,4-sulfonyldianiline

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-21 Bioassays of Aldrin and Dieldrin for Possible Carcinogenicity (CAS No. 309-00-2) (CAS No. 60-57-1)

Aldrin and dieldrin are organochlorine insecticides of the cyclodiene group. These chemicals are neurotoxins, and their predominant effect is the stimulation of the nervous system. Both aldrin and dieldrin are lipophilic and accumulate in mammalian tissues. Aldrin undergoes metabolic conversion to the epoxide, dieldrin, and because of this structural relationship, reports of the bioassays of both chemicals have been combined in this single report.

Bioassays of technical-grade aldrin and dieldrin for possible carcinogenicity were conducted by administering the test materials in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Aldrin

Groups of 50 rats of each sex were administered aldrin at one of two doses, either 30 or 60 ppm. Male rats were treated for 74 weeks, followed by 37-38 weeks of observation; female rats were treated for 80 weeks, followed by 32-33 weeks of observation. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 58 untreated males and 60 untreated females from similar bioassays of other chemicals. All surviving rats were killed at 111-113 weeks.

Groups of 50 mice of each sex were administered aldrin at one of two doses for 80 weeks, then observed for 10-13 weeks. Time-weighted average doses were 4 or 8 ppm for males and 3 or 6 ppm for females. Matched controls consisted of groups of 20 untreated male mice and 10 female mice; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 92 untreated male and 79 untreated female mice from similar bioassays of other chemicals. All surviving mice were killed at 90-93 weeks.

Mean body weights attained by the rats and mice fed diets containing aldrin were similar to those of the controls during the first year of the study; however, mean body weights of the treated rats were lower than those of the controls during the second year of the study. Hyperexcitability was observed in all treated groups with increasing frequency and severity during the second year. Aldrin produced no significant effect on the mortality of rats or of male mice, but there was a dose-related trend in the mortality of female mice, primarily due to the early deaths in the high-dose groups.

There was an increased combined incidence of follicular-cell adenoma and carcinoma of the thyroid both in male rats fed aldrin (matched controls 3/7, pooled controls 4/48, low-dose 14/38, high-dose 8/38) and female rats fed aldrin (matched controls 1/9, pooled controls 3/52, low-dose 10/39, high-dose 7/46). These incidences were significant in the low-dose but not in the high-dose groups both of males ($P = 0.001$) and females ($P = 0.009$) when compared with the pooled controls. Comparisons with matched controls, however, were not significant.

Cortical adenoma of the adrenal gland was also observed in aldrin-treated rats in significant proportions ($P = 0.001$) in low-dose (8/45) but not in high-dose (1/48) females when compared with pooled controls (0/55). Because these increased incidences were not consistently significant when compared with matched rather than pooled control groups, it is questionable whether the incidences of any of these adrenal tumors were associated with treatment.

In male mice, there was a significant dose-related increase in the incidence of hepatocellular carcinomas (matched controls 3/20, pooled controls 17/92, low-dose 16/49, high-dose 25/45) when compared with either matched controls ($P = 0.001$), or pooled controls ($P < 0.001$). The incidence in the high-dose group was significant when compared with matched controls ($P = 0.002$) or pooled controls ($P < 0.001$).

Dieldrin

Groups of 50 rats and 50 mice of each sex were administered dieldrin at one of two doses. Low-dose rats and both low- and high-dose mice were treated for 80 weeks, followed by observation periods of 30-31 weeks for rats and 10-13 weeks for mice. Treatment of high-dose rats was terminated after 59 weeks and followed by 51-52 weeks of observation. Time-weighted average doses for rats were 29 or 65 ppm; doses for mice were 2.5 or 5 ppm. Matched controls consisted of groups of 10 untreated rats of each sex and 20 untreated male mice and 10 female mice; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with untreated animals from similar bioassays of other chemicals (58 male and 60 female rats, 92 male and 79 female mice). All surviving rats were killed at 110-111 weeks, and all surviving mice at 90-93 weeks.

Mean body weights attained by the rats and mice fed diets containing dieldrin showed little or no differences compared with those of the controls during the first year of the study; however, mean body weights of the treated rats were lower than those of the controls during the second year of the study. Hyperexcitability was observed in all treated groups with increasing frequency during the second year, especially in high-dose rats.

There was a marked increase in the mortality rate of rats during the first 90 weeks of the study. However,

because of the high rates of mortality in the control groups during the remaining 20 weeks, survival could not be shown to be statistically dose responsive.

In rats, there was a significant (P = 0.007) difference between the combined incidence of adrenal cortical adenoma or carcinoma in the low-dose females (6/45) and that in the pooled controls (0/55). Although this tumor was also found in animals treated with aldrin, it is not clearly associated with treatment, because the incidence in the high-dose (2/40) was not significant, and the incidences were not significant when matched, rather than pooled, controls were used for comparison.

In male mice, there was a significant positive dose-related trend (P = 0.020) in the incidence of hepatocellular carcinomas using the pooled controls (pooled controls 17/92, low-dose 12/50, high-dose 16/45). When high-dose males were compared with the pooled controls, the results were also significant (P = 0.025).

It is concluded that under the conditions of these bioassays, none of the tumors occurring in Osborne-Mendel rats treated with aldrin or dieldrin could clearly be associated treatment.

Aldrin was carcinogenic for the liver of male $B6C3F_1$ mice producing hepatocellular carcinomas. With dieldrin, there was a significant increase in the incidence of hepatocellular carcinomas in the high-dose males which may be associated with treatment.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

For Aldrin:	
Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Negative
For Dieldrin:	
Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

Note: Dieldrin was also studied by administration in feed to F344 rats (See TR-022, reported 1978).

TR-22 Bioassay of Dieldrin for Possible Carcinogenicity (CAS No. 60-57-1)

Dieldrin is a chlorinated cyclodiene pesticide. It is also a metabolic conversion product of aldrin, another pesticide, and can be expected to appear in the environment following the use of either chemical. Dieldrin was first introduced in the 1950's for use by cotton growers when the chemical was found to be more effective than aldrin, and later, was used as an insecticide for other crops, for public health pest control, and for mothproofing woolen goods.

A bioassay of purified technical-grade dieldrin for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered dieldrin at one of three doses, either 2, 10, or 50 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks.

Body weights of the rats were essentially unaffected by the treatment, but typical signs of organochlorine intoxication including hyperexcitability, tremors, and coma were observed in high-dose males beginning in week 76 and in high-dose females beginning in week 80. Survival was not adversely affected, and adequate numbers of rats were available for meaningful statistical analyses of the incidences of tumors.

A variety of neoplasms occurred in control and treated rats; however, the incidences were not related to treatment.

It is concluded that under the conditions of this bioassay, dieldrin was not carcinogenic in Fischer 344 rats.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative

Note: Dieldrin was also studied in feed in Osborne-Mendel rats and $B6C3F_1$ mice (See TR-21, reported 1978).

TR-23 Bioassay of Picloram for Possible Carcinogenicity (CAS No. 1918-02-1)

Picloram is a systemic herbicide registered by EPA for only nonfood use to control broadleaf weeds and woody plants. The chemical can replace the plant growth hormone indoleacetic acid, and inhibit the synthesis of protein in plants.

A bioassay of technical-grade picloram for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered picloram in the diet at one of the following doses for 80 weeks. Time-weighted average doses for the rats were 7,437 or 14,875 ppm; those for the mice were 2,531 or 5,062 ppm. The rats were then observed for 33 weeks, the mice for 10 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched control groups combined with 33 untreated male and 30 untreated female rats or mice from similar bioassays of three other test chemicals. All surviving rats were killed at 113 weeks; all surviving mice were killed at 90 weeks. Survival was adequate for meaningful statistical analyses of the incidences of tumors in rats and mice of both sexes.

Mean body weights of the high-dose rats were lower than those of matched controls during the first part of the study; however, beginning at approximately 80 weeks, mean weights of controls were lower than those of treated animals. Body weights of the mice were unaffected by the picloram.

In rats, a relatively high incidence of follicular hyperplasia, C-cell hyperplasia, and C-cell adenoma of the thyroid occurred in both sexes. However, the statistical tests for adenoma did not show sufficient evidence for association of the tumor with picloram administration.

An increased incidence of hepatic neoplastic nodules was observed in treated male and female rats as compared with untreated animals. This lesion is considered to be a benign tumor. In male rats the lesion appeared only in three animals of the low-dose treatment group and was not significant when compared with the controls; however, the test for positive dose-related trend in females was significant (pooled controls 0/39, low-dose 5/50, high-dose 7/49, $P = 0.016$) and the incidence in the high-dose group was significant ($P = 0.014$) when compared with that in the pooled-control group.

There was also one hepatocellular carcinoma in a low-dose male rat and one in a high-dose female rat. In both males and females, there was a possibly treatment-related lesion of the liver diagnosed as foci of cellular alteration. The incidences of this latter lesion were, female rats: matched controls 1/10, low-dose 8/50, high-dose 18/49; male rats: matched controls 0/10, low-dose 12/49, high-dose 5/49. Thus, there is evidence that picloram affected the livers of rats of both sexes, but more particularly those of the females.

No tumors were found in male or female mice or male rats at incidences that could be significantly associated with treatment, and it is concluded that picloram was not carcinogenic for $B6C3F_1$ mice or male Osborne-Mendel rats.

In female rats, however, the incidence of neoplastic nodules of the liver, benign tumors, was associated with treatment with picloram. It is concluded that under the conditions of the bioassay, the findings are suggestive of the ability of the compound to induce benign tumors in the livers of female Osborne-Mendel rats.

Synonym: 4-amino-3,5,6-trichloropicolinic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-24 Bioassay of Malathion for Possible Carcinogenicity (CAS No. 121-75-5)

Malathion is an organophosphorus insecticide and acaricide first synthesized in the United States in 1952. Malathion primarily affects the nervous system by inhibition of cholinesterase activity and subsequent accumulation of acetylcholine. However, it has a low mammalian toxicity. Malathion is approved for a wide variety of uses as an insecticide and acaricide on field crops, fruits, nut trees, vegetables, livestock, agricultural premises, and land. Tolerances for residues of malathion have been established on many of these products.

A bioassay of technical-grade malathion for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered malathion at one of two doses for 80 weeks, then observed for 33 weeks. Time-weighted average doses were 4,700 or 8,150 ppm. Matched controls consisted of groups of 15 untreated rats of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-113 weeks.

Groups of 50 mice of each sex were administered malathion at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 94 or 95 weeks.

Mortality in either rats or mice was not significantly related to the administration of malathion. Sufficient numbers of animals were at risk in the dosed and control groups of rats and mice of each sex for development of late-appearing tumors.

In female rats, three follicular-cell carcinomas and one follicular-cell adenoma of the thyroid occurred in the high-dose group, and three follicular-cell hyperplasias occurred in the low-dose group. The incidence of these tumors showed a statistically significant ($P = 0.026$) dose-related trend; however, the results of the Fisher exact test for direct comparison between the dosed and control groups were not significant. More dosed males than dosed females had either tumors or hyperplasia of the follicular cells of the thyroid; however, because of the higher incidence of tumors among the male controls, none of the results of the statistical tests were significant. These thyroid tumors were not considered to be associated with the administration of malathion.

In male mice, hepatocellular carcinoma occurred at the following incidences: matched controls 2/10, pooled controls 5/49, low-dose 7/48, high-dose 11/49. In addition, neoplastic nodules occurred in 3/49 pooled-control and 6/49 high-dose animals. When the combined incidence of these neoplasms in the dosed animals was compared with that of the pooled controls, the dose-related trend was $P = 0.019$ and the direct comparison of the high-dose group with the control group was $P = 0.031$. Thus, none of the direct comparisons of dosed groups with controls were significant using the Bonferroni criteria. In addition, the historical controls from this laboratory had several control groups with incidences of 35-40% hepatocellular carcinoma, rates which are comparable with the incidence of this tumor in the dosed male mice of the present study. Thus, these liver tumors are not considered to be associated with the administration of malathion. The incidences of liver tumors in dosed females were not statistically

significant when compared with that in control animals.

It is concluded that under the conditions of this bioassay, there was no clear evidence of the association of the tumor incidence with the administration of malathion to Osborne-Mendel rats or B6C3F$_1$ mice.

Synonym: S-(1,2-dicarbethoxyethyl)-*O,O*-dimethyldithiophosphate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

Note: Malathion was subsequently studied by administration in feed to F344 rats (See TR-192, reported 1979).

TR-25 Bioassay of Chloramben for Possible Carcinogenicity (CAS No. 133-90-4)

Chloramben has been used since 1958 as a preemergent herbicide to control shallow, germinating, broadleaf weeds and annual grasses. Applied as a spray at the time of planting, chloramben remains effective in the soil for several weeks until crops have become well established.

A bioassay of technical-grade chloramben for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and B6C3F$_1$ mice. Groups of 50 rats and 50 mice of both sexes were administered chloramben at one of two doses, either 10,000 or 20,000 ppm. The rats were treated for 80 weeks, then observed for 32 or 33 weeks; the mice were treated for 80 weeks, then observed for 11 or 12 weeks. Matched controls consisted of groups of 10 untreated rats and 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of these matched controls combined with 75 untreated male and 75 untreated female rats or 70 untreated male and 70 untreated female mice from similarly performed bioassays of six other test chemicals. Surviving rats were killed at 112 or 113 weeks; surviving mice were killed at 91 or 92 weeks.

Body weights and mortality of the treated animals were not markedly affected by chloramben under the conditions of the bioassay. The various clinical signs observed were common to both treated and control groups.

In male rats, hemangiomas occurred at a significantly higher incidence in the low-dose animals than in the pooled controls (controls 0/73, low-dose 5/48, $P = 0.009$). This lesion was not considered to be related to the administration of chloramben, since the tumor did not occur at a significantly higher incidence in the high-dose group than in the pooled-control group, and the incidences did not show a significant dose-related trend.

In both male and female mice, the incidences of hepatocellular carcinoma showed significant dose-related trends using pooled controls (for females: controls 9/69, low dose 16/48, high-dose 14/48, $P = 0.029$; for females controls 2/67, low-dose 7/48, high-dose 10/50, $P = 0.004$). Direct comparisons showed significantly higher incidences of the tumor in the low-dose males ($P = 0.008$) and in the high-dose females ($P = 0.003$) than in the pooled controls. Probability levels of $P = 0.028$ in high-dose males and $P = 0.027$ in low-dose females were attained. In male mice, however, the incidence of hepatocellular carcinoma was considered to be only marginally associated with the administration of chloramben because of the variations in the spontaneous incidence of this lesion in male mice encountered at this laboratory.

In conclusion, under the conditions of this bioassay, there were no tumors in Osborne-Mendel rats that were significantly related to administration of the chemical. In B6C3F$_1$ female mice, chloramben was carcinogenic, producing hepatocellular carcinomas in treated animals.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Positive

TR-26 Bioassay of Nitrofen for Possible Carcinogenicity (CAS No. 1836-75-5)

Nitrofen, a substituted diphenyl ether, is one of several agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data.

A bioassay of technical-grade nitrofen for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Nitrofen was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of nitrofen were 3,656 and 2,300 ppm for male rats, 2,600 and 1,300 ppm for female rats, and 4,696 and 2,348 ppm for both male and female mice, respectively. After a 78-week treatment period, observation of the low dose and control male and all female rats continued for an additional 32 weeks; observation of the high dose male rats continued for an additional 4 weeks. All mice were observed for an additional 12 weeks after the 78-week treatment period.

For each species, 20 animals of each sex were placed on test as controls. No nitrofen was added to their diet.

The incidence of carcinomas of the pancreas had a statistically significant positive association with concentration of nitrofen in the diet of female rats. The incidence of this tumor in high dose female rats was significant when compared to controls. Poor survival related to chemical toxicity precluded the evaluation of the carcinogenicity of nitrofen in male rats.

In mice of both sexes, the incidence of hepatocellular carcinoma at both high and low dose levels was highly significant when compared to the controls. The incidence of hemangiosarcoma of the liver had a statistically signifi-

cant relationship with nitrofen concentration in the diet for mice of both sexes, and the incidence in high dose male mice was significant when compared to controls.

The results of this study indicate that orally administered technical-grade nitrofen is a liver carcinogen in B6C3F$_1$ mice of both sexes. Nitrofen is also carcinogenic to female Osborne-Mendel rats.

Synonyms: 2,4-dichloro-1-(4-nitrophenoxy)-benzene; 2,4-dichlorophenyl-p-nitrophenyl ether; nitrophene, Tok E-25; Nip

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

Note: Nitrofen was subsequently studied by administration in feed to F344 rats and B6C3F$_1$ mice (See TR-184, reported 1979).

TR-27 Bioassay of 1,1,2,2-Tetrachloroethane for Possible Carcinogenicity (CAS No. 79-34-5)

1,1,2,2,-Tetrachloroethane, an aliphatic chlorinated hydrocarbon, is one of a group of halogenated solvents selected for bioassay by the National Cancer Institute. Solvents were selected on the basis of large-scale production, extensive use, and lack of adequate chronic toxicity data.

A bioassay for possible carcinogenicity of technical-grade 1,1,2,2-tetrachloroethane was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. At initiation of the study the rats were approximately 7 weeks old, and the mice were approximately 5 weeks old. 1,1,2,2-Tetrachloroethane in corn oil was administered by gavage, at either of two dosages, to two groups of 50 male and 50 female animals of each species, 5 days a week. Treatment was over a period of 78 weeks, followed by observation periods of 32 weeks for the rats and 12 weeks for the mice. The high and low time-weighted average dosages were, respectively, 108 and 62 mg/kg/day for male rats, 76 and 43 mg/kg/day for female rats, and 282 and 142 mg/kg/day for the mice of both sexes.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were intubated with corn oil at the same rate as the high dose animals. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

Among mice, hepatocellular carcinomas were observed in 2/16 (13 percent) male untreated controls, 1/18 (6 percent) male vehicle controls, 13/50 (26 percent) low dose males, 44/49 (90 percent) high dose males, 0/18 female untreated controls, 0/20 female vehicle controls, 30/48 (63 percent) low dose females, and 43/47 (91 percent) of the high dose females. This incidence of hepatocellular carcinoma indicated a highly significant ($P<0.001$) positive dose-related trend in mice of both sexes.

No statistically significant incidence of neoplastic lesions was observed in male or female rats. However, two hepatocellular carcinomas and one neoplastic nodule, which are rare tumors in the male Osborne-Mendel rat, were observed in the high dose males.

Under the conditions of this study, orally administered 1,1,2,2-tetrachloroethane is a liver carcinogen in B6C3F$_1$ mice of both sexes. The results do not provide conclusive evidence for the carcinogenicity of 1,1,2,2-tetrachloroethane in Osborne-Mendel rats.

Synonyms: acetylene tetrachloride; sym-tetrachloroethane

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-28 Bioassay of Dibromochloropropane for Possible Carcinogenicity (CAS No. 1836-75-5)*

Dibromochloropropane, a halogenated aliphatic hydrocarbon and agricultural pesticide, was one of several widely used pesticides selected for bioassay by the National Cancer Institute. At this time there was a lack of adequate chronic toxicity data on this compound.

A bioassay for possible carcinogenicity of technical-grade dibromochloropropane (DBCP) was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. DBCP in corn oil was administered by gavage 5 days a week, at either of two dosages, to groups of 50 male and 50 female animals of each species.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average dosages of DBCP in the chronic study were 29 mg/kg/day for the high dose rats of both sexes, and 15 mg/kg/day for the low dose rats of both sexes. The time-weighted average concentrations for the high dose male and female mice were 219 and 209 mg/kg/day, respectively. The time-weighted average concentrations for the low dose male and female mice were 114 and 110 mg/kg day, respectively.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were intubated with corn oil at the same time that dosed animals were intubated with DBCP mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals received no gavage treatments.

DBCP was administered to the high dose male and high dose female rats for 64 weeks prior to sacrifice, and to the low dose female rats for 73 weeks prior to sacrifice. The low dose male rats were treated for 78 weeks followed by an additional 5 weeks of observation. The high dose male and female mice were treated for 47 weeks prior to sacrifice; the low dose male mice were treated for 59 or 60 weeks prior to sacrifice, and the low dose female mice were treated for 60 weeks prior to sacrifice.

In rats and mice of both sexes, statistically significant incidences of squamous-cell carcinomas of the forestomach occurred in each dosed group and a significant positive association existed between dosage level and tumor incidence. The incidences of adenocarcinomas of the mammary gland were statistically significant in female rats when the treated groups were compared to the controls. Toxic nephropathy was also observed at elevated incidences in all of the treated rats and mice when compared to their respective untreated or vehicle control groups.

Under the conditions of this study, DBCP is a stomach carcinogen in rats and mice of both sexes and is carcinogenic to the mammary gland in female rats.

Synonyms: 1,2-dibromo-3-chloropropane; Nemagon®; Fumazone®; DBCP

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

*The technical report states that the Chemical Abstract Service (CAS) Ninth Collective Index (1977) name for this compound is 1,2-dibromo-3-chloropropane (CAS No. 96-12-8). This is the CAS number used to track this study in the NTP CHEMTRACK database.

Note: Dibromochloropropane was subsequently studied by inhalation to F344 rats and B6C3F$_1$ mice (See TR-206, reported 1982).

TR-29 Bioassay of 2-Methyl-1-Nitroanthraquinone for Possible Carcinogenicity (CAS No. 129-15-7)

2-Methyl-1-nitroanthraquinone, an intermediate in the synthesis of anthraquinone dyes, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer among workers in the dye manufacturing industry. Aromatic nitro compounds are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry.

A bioassay of 2-methyl-1-nitroanthraquinone for possible carcinogenicity was conducted using Fischer 344 rats. 2-Methyl-1-nitroanthraquinone was administered in the feed at either of two concentrations to groups of 50 male and 50 female animals. The high and low dietary concentrations used were 0.12 and 0.06 percent, respectively, for the male and female rats. After a 78-week treatment period, observation of the rats continued for an additional 31 weeks. Fifty rats of each sex were placed on test as controls. No 2-methyl-1-nitroanthraquinone was added to their diet.

Survival in both the male and female rats was adequate for a meaningful statistical analysis of late-developing tumors; however, there was a significant positive association between increased dosage and elevated mortality in female rats.

Hepatocellular carcinomas and neoplastic nodules of the liver occurred in both the male and female treated rats. A statistically significant association between increased dosage and an elevated incidence of hepatocellular carcinomas was indicated by the Cochran-Armitage test for the males (1/48, 5/48, and 9/49 in control, low dose, and high dose, respectively); however, the Fisher exact tests supported these results only for the high dose males. The incidence of neoplastic nodules was statistically significant in the male rats (0/48, 2/48, and 6/49 in control, low dose, and high dose, respectively), as indicated by the Cochran-Armitage test and supported by the Fisher exact test for the high dose group. When those rats having either hepatocellular carcinomas or neoplastic nodules of the liver were combined and evaluated simultaneously, the Cochran-Armitage tests indicated statistically significant associations between increased dosages and elevated tumor incidences in both the males and females. This was supported by the Fisher exact tests for males but not for females. The incidences of one tumor type, subcutaneous fibroma, were found to be statistically significant in both male and female rats. No other tumors occurred in treated animals in statistically significant incidences when compared to controls.

Squamous-cell papillomas and squamous cell carcinomas of the forestomach were observed only in high dose rats. Although the incidences of these gastric tumors were not statistically significant, historical data indicate that these tumors are rare in Fischer 344 rats. The occurrence of these tumors in high dose rats, together with the frequent occurrence of nonneoplastic proliferative lesions of the forestomach in treated rats, indicates that the occurrence of these tumors was related to administration of 2-methyl-1-nitroanthraquinone. An increased incidence of bladder tumors (papillomas, transitional-cell papillomas, and sarcomas) was observed among female rats.

Under the conditions of this bioassay, the results indicate that orally administered 2-methyl-1-nitroanthraquinone is carcinogenic in male Fischer 344 rats, producing hepatocellular carcinomas. Increased incidences of subcutaneous fibromas in both male and female Fischer 344 rats were also associated with the administration of the compound. Tumors of the forestomach and bladder in these animals may also have been related to the administration of the test chemical.

Synonyms: 2-methyl-1-nitro-9,10-antracenedione; 1-nitro-2-methyl-anthraquinone

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-30 Bioassay of Diarylanilide Yellow for Possible Carcinogenicity (CAS No. 6358-85-6)

Diarylanilide yellow, one member of a family of organic azo pigments known as benzidine yellows, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those dyes and dye intermediates which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay of technical-grade diarylanilide yellow for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. Diarylanilide yellow was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low dietary concentrations used in the chronic study for the male and female rats and mice were 5.0 and 2.5 percent, respectively, of the chemical in the feed. After a 78-week treatment period, observation of the rats continued for an additional 28 weeks and observation of the mice continued for an additional 19 weeks for high dose males and low and high dose females and 18 weeks for low dose males. For each species, 50 animals of each sex were placed on test as controls, and fed only the basal diet.

The high concentration administered to both species in this study was the maximum recommended in the Guidelines for Carcinogen Bioassay in Small Rodents. These guidelines indicate that a chronic dietary level of 5 percent, or 50,000 ppm, should not be exceeded even when no signs of toxicity are observed during subchronic testing, except under special circumstances (e.g., when the compound is a major component of the human diet). No toxic effects were reported during subchronic testing and diarylanilide yellow did not qualify for exception; therefore, the highest permissible concentration (5 percent) was utilized in the chronic bioassay.

The dietary concentrations of diarylanilide yellow administered during the chronic bioassay had no significant effect on survival or body weight gain in either species. Except for yellow staining and some isolated neoplasms, the only adverse clinical sign or pathologic lesion observed in treated rats or mice was basophilic cytoplasm changes in hepatocytes of treated rats.

In both species the survival in all groups was adequate for statistical analysis of late-appearing tumors.

No treatment-related increase in the incidence of neoplasms or nonneoplastic lesions was evident in treated rats or mice. A few unusual findings were observed in both species, including single cases of metastatic chordoma and osteogenic sarcoma in rats, and single cases of squamous-cell carcinoma of the ear, infiltrating duct carcinoma of the mammary gland, and subcutaneous mastocytoma in mice.

The results of the study did not provide evidence for the carcinogenicity of diarylanilide yellow in Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: 2,2'-[(3,3'-dichloro(1,1'-biphenyl)-4,4'-diyl)-bis (azo)] bis(3-oxo-N-phenyl)-butanamide; C.I. Pigment Yellow 12; Diarylide yellow

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-31 Bioassay of Tolbutamide for Possible Carcinogenicity (CAS No. 64-77-7)

Tolbutamide was the first oral hypoglycemic agent used in the management of diabetes. It is one of the arylsulfonylurea hypoglycemics, a group which included tolazamide, chlorpropamide, and acetohexamide. All of these compounds function by stimulating the secretion of insulin by the pancreas and, therefore, are used only in patients with at least minimal pancreatic function, as in maturity-onset diabetics.

A bioassay of tolbutamide for possible carcinogenicity was conducted by administering the test material in the diet to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 35 rats of each sex were administered tolbutamide at one of two doses, either 12,000 or 24,000 ppm, 5 days a week for 78 weeks, then observed for an additional 28 weeks. Matched-control groups consisted of 15 untreated rats of each sex. All surviving rats were killed at 106 or 107 weeks.

Groups of 35 mice of each sex were administered tolbutamide at one of two doses, either 25,000 or 50,000 ppm, 5 days a week for 78 weeks, then observed for an additional 24-26 weeks. Matched-control groups consisted of 15 untreated mice of each sex. All surviving mice were killed at 102-104 weeks.Mean body weights of the treated rats and mice were lower than those of the corresponding matched controls during the entire study; however, survival was not significantly affected by treatment in either species. In both sexes of both species, survival was considered to be adequate for meaningful statistical analyses of the incidence of tumors.

In both the rats and the mice, a variety of neoplasms were found in both tolbutamide-treated and control groups. None of the neoplasms were present at a statistically significant increased incidence in treated groups of either species as compared with control groups and were not considered to be compound related.

It is concluded that under the conditions of this bioassay, tolbutamide was not carcinogenic for either Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 1-butyl-3-(p-methylbezenesulfonyl)urea

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-32 Bioassay of Isophosphamide for Possible Carcinogenicity (CAS No. 3778-73-2)

A bioassay of the anticancer drug isophosphamide for possible carcinogenicity was conducted by injecting the test chemical intraperitoneally into Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were given the injections at one of two doses three times per week for 52 weeks. Doses for rats were either 6 or 12 mg/kg, and for mice either 10 or 20 mg/kg. After the period of administration of the isophosphamide, the surviving rats were observed for 31 weeks and the mice for 28 weeks. Untreated controls as well as vehicle controls (buffered saline) were used. The matched vehicle-control groups each consisted of 10 rats or 15 mice of each sex; pooled vehicle-control groups, used for statistical evaluation, consisted of the matched vehicle controls of each species combined with 20 male and 20 female matched vehicle-control rats or 15 male and 15 female matched vehicle-control mice from a similar bioassay of another test chemical. All surviving rats were killed at 79-84 weeks, all surviving mice at 79-81 weeks.

Mean body weights of the high-dose rats of either sex were lower than those of the matched vehicle controls after approximately 25 weeks on study. Survival was low among the high-dose male and female rats, but in the low-dose groups it was adequate for meaningful statistical analyses of the incidences of tumors. Mean body weights of the mice did not show any consistent effect from the isophosphamide treatment. Survival was adequate for meaningful statistical analyses in both groups of female mice, while survival of the males was 31% for both treated groups at the end of the study.

In male rats, tumors of the hematopoietic system included six malignant lymphomas and two granulocytic leukemias. With the unadjusted analysis, these tumors showed a dose-related trend in male rats using pooled vehicle controls (controls 0/29, low-dose 3/32, high-dose 5/34, $P = 0.032$) and a higher incidence in the high-dose males than in the pooled vehicle controls ($P = 0.040$). These tumors were not significant when compared with matched vehicle controls using adjusted analyses, and they cannot clearly be associated with treatment. However, it should be noted that five rats with these tumors were observed in the high-dose group whose median survival was only 35 weeks.

In female rats, the incidence of uterine leiomyosarcoma was significant in the low-dose group using pooled vehicle controls (controls 0/27, low-dose 15/32, $P<0.001$). There was also a significant incidence of mammary fibroadenoma among low-dose females using pooled vehicle controls (controls 8/28, low-dose 28/33, $P<0.001$). The incidence of each tumor was also significant when compared with matched vehicle controls using time-adjusted analyses. The low survival of the high-dose group (median time on study, 35 weeks) may explain the lower incidences of the uterine leiomyosarcoma and the mammary fibroadenoma in this group. In some rats, the leiomyosarcomas metastasized to the lungs, urinary bladder, spleen, and other abdominal sites.

In female mice, the incidence of malignant lymphoma of the hematopoietic system showed a significant dose-related trend using either matched vehicle controls (controls 0/14, low-dose 3/32, high-dose 13/34, $P=0.001$) or pooled vehicle controls (controls 1/29, $P<0.001$). Further, incidences of this tumor in the high-dose females were significantly higher than incidences in the matched vehicle ($P=0.005$) or pooled vehicle ($P=0.001$) controls.

It is concluded that under the conditions of this bioassay, isophosphamide was not carcinogenic in male Sprague-Dawley rats or in male $B6C3F_1$ mice. However, the incidence of leiomyosarcomas of the uterus indicates that isophosphamide was carcinogenic in female Sprague-Dawley rats, and the incidence of fibroadenoma of the mammary gland in female rats was associated with treatment with isophosphamide. Isophosphamide was carcinogenic in female $B6C3F_1$ mice, producing malignant lymphomas of the hematopoietic system.

Synonyms: 3-(2-chloroethyl)-2-[2-chloroethyl)amino]-tetrahydro-1,3,2-oxazaphosphorine-2-oxide; ifosfamide

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-33 Bioassay of Tetrachlorvinphos for Possible Carcinogenicity (CAS No. 961-11-5)

Tetrachlorvinphos is an organophosphorous pesticide introduced in 1966 by Shell Development Company. It is registered for use against various pests of fruits, vegetables, ornamental plants, forest trees, and livestock, and for use on agricultural premises, agricultural equipment, and recreational areas.

A bioassay of technical-grade tetrachlorvinphos for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered tetrachlorvinphos at one of two doses for 80 weeks, then observed for 31 additional weeks. Time-weighted average doses were either 4,250 or 8,500 ppm. Matched controls

consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 45 untreated male and 45 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 111 weeks.

Groups of 50 mice of each sex were administered tetrachlorvinphos at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 12 additional weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-92 weeks.

The mean body weights of the treated rats and mice were generally lower than those of the matched controls; however, the mortality rate was affected adversely by tetrachlorvinphos only in the male rats. Survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors, except for a matched-control group of female rats for which the survival was abnormally low.

In rats, C-cell adenoma of the thyroid showed a significant dose-related trend in the females, using pooled controls (controls 1/46, low-dose 2/50, high-dose 7/46, $P = 0.013$), and by direct comparison, an increased incidence in the high-dose group ($P = 0.027$). High incidences of C-cell hyperplasia in treated males and females further indicated a chemical-related effect on proliferative lesions of the thyroid. Cortical adenoma of the adrenal also showed a significant dose-related trend in the females, using pooled controls (controls 0/50, low-dose 2/49, high-dose 5/50, $P = 0.017$), and by direct comparison, an increased incidence in the high-dose group ($P = 0.022$). Hemangioma of the spleen occurred in male rats at a significantly higher incidence in the low-dose group than in the pooled controls (controls 0/52, low-dose 4/48, $P = 0.049$); however, neither the incidence in the high-dose group (0/47) nor the test result for dose-related trend was statistically significant.

In mice, hepatocellular carcinoma in males showed a highly significant dose-related trend, using either matched controls (controls 0/9, low-dose 36/50, high-dose 40/50, $P < 0.001$) or pooled controls (controls 5/49, $P < 0.001$). This finding was supported by direct comparisons of low- and high-dose groups of males with matched- or pooled-control groups, which showed highly significant increases in incidences of the tumor in the treated groups in all instances ($P < 0.001$). In females, the incidence of hepatocellular carcinoma was not significant; however, the incidence of neoplastic nodule was significantly higher in both the low- and high-dose groups than in the pooled controls (controls 1/48, low-dose 14/49, $P < 0.001$; high-dose 9/47, $P = 0.007$), using pooled controls for tests for both doses. Because of this higher incidence in the low-dose group than in the high-dose group, there was a significant departure from linear trend ($P = 0.006$).

Granulomatous lesions of the liver were found in high proportions in both treated rats and treated mice, but none were found in matched controls.

It is concluded that under the conditions of this bioassay, the administration of technical-grade tetrachlorvinphos in Osborne-Mendel rats was associated with proliferative lesions of the C cells of the thyroid and cortical adenomas of the adrenal in females. In female $B6C3F_1$ mice, the incidence of neoplastic nodule of the liver was associated with treatment, and in male mice tetrachlorvinphos was carcinogenic, causing hepatocellular carcinoma of the liver.

Synonym: 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-34 Bioassay of Trifluralin for Possible Carcinogenicity (CAS No. 1582-09-8)

Trifluralin, a tertiary aromatic amine and dinitrotoluene derivative, is one of several widely used agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data.

A bioassay for possible carcinogenicity of technical-grade trifluralin was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Analysis of the technical product established the presence of 84 to 88 ppm dipropylnitrosoamine. The product was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Fifty animals of each sex were placed on test as controls for the rat bioassay, while 20 of each sex were utilized as controls for the mouse study. The time-weighted average high and low dietary concentrations of trifluralin were, respectively, 8,000 and 4,125 ppm for male rats, 7,917 and 4,125 ppm for female rats, 3,744 and 2,000 ppm for male mice, and 5,192 and 2,740 ppm for female mice. After a 78-week treatment period, there was an additional observation period of 33 weeks for rats and 12 weeks for mice.

For female mice the association between increased dosage and elevated incidence of hepatocellular carcinomas was significant (0/20, 12/47, and 21/44 of the control, low dose, and high dose, respectively) as was the relationship between dose and incidence of alveolar/bronchiolar adenomas. Significance of incidence for both types of tumors was supported by tests for significance at each dose level. Squamous-cell carcinomas of the stomach were observed in dosed female mice, but not in controls. Although incidences of these tumors were not statistically significant, they are unusual lesions in $B6C3F_1$ mice and are considered to be treatment-related.

Neoplasms observed in treated rats were types that have occurred spontaneously in this strain and were apparently unrelated to trifluralin treatment.

Evaluation of the results of this bioassay indicates that

technical-grade trifluralin is a carcinogen in female B6C3F$_1$ mice, being associated in these animals with an elevated incidence of hepatocellular carcinomas, alveolar/bronchiolar adenomas and squamous-cell carcinomas of the forestomach. Sufficient evidence was not provided for the carcinogenicity or tumorigenicity of trifluralin in male B6C3F$_1$ mice or in Osborne-Mendel rats of either sex.

Synonyms: 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzeneamine; α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; N,N,-dipropyl-4-trifluoromethyl-2,6-dinitroaniline; Treflan

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Positive

TR-35 Bioassay of Methoxychlor for Possible Carcinogenicity (CAS No. 72-43-5)

Methoxychlor, a synthetic organochlorine insecticide and structural analog of DDT, was one of several widely used agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data. The suspect state of all DDT-related chemicals was an important additional factor in its selection.

A bioassay for possible carcinogenicity of technical-grade methoxychlor was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Methoxychlor was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For each species, 20 animals of each sex were placed on test as controls. The time-weighted average high and low dietary concentrations of methoxychlor were, respectively, 845 and 448 ppm for male rats, 1,385 and 750 ppm for female rats, 3,491 and 1,746 ppm for male mice, and 1,994 and 997 ppm for female mice. After a treatment period of 78 weeks, the rat groups were observed for an additional 34 weeks and the mouse groups for an additional 15 weeks. A dose-related mean group body weight depression was observed in both rats and mice, but no effect on survival was detected.

Under the conditions of this study, methoxychlor was not found to be carcinogenic in Osborne-Mendel rats or B6C3F$_1$ mice of either sex.

Synonyms: 1,1'-(2,2,2-trichloroethylidene)bis (4-methoxy)-benzene; 1,1,1-trichloro-2,2'-bis(p-methoxyphenyl)-ethane; dianisyl trichloroethane; Dimethoxy DDT; DMDT

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-36 Bioassay of Anthranilic Acid for Possible Carcinogenicity (CAS No. 118-92-3)

Anthranilic acid is an aromatic amine which occurs physiologically as a metabolite of the amino acid tryptophan. It is used commercially as an intermediate in dye synthesis.

A bioassay of anthranilic acid for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered anthranilic acid at one of the following doses, either 15,000 or 30,000 ppm for the rats, and either 25,000 or 50,000 ppm for the mice, 5 days per week for 78 weeks, then observed for an additional 26-27 weeks. Matched controls consisted of groups of 15 rats and 15 mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 15 untreated male and 15 untreated female animals of each species from a similar bioassay of another test chemical Except for the matched-control male mice, all surviving animals in the study were killed at 104-106 weeks. Half of the matched-control male mice, which were accidentally killed at week 12, were excluded from the report; the remaining matched-control males died by week 94.

Mean body weights of the low- and high-dose male and high-dose female rats were lower than those of the corresponding matched controls for the duration of the study. The weights of the low-dose females were similar to those of the matched controls for the first 45 weeks, after which they declined slightly. The weights of the low-dose male mice were similar to those of the matched controls, while those of the high-dose males and of the low- and high- dose females were slightly lower.

Survival of both treated and matched-control groups of rats of both sexes was high; survival of treated mice of both sexes and of female matched controls, although lower than that of the rats, was sufficient for meaningful statistical analyses of the incidences of tumors.

In rats, a variety of neoplasms were observed in both treated and control animals. Few malignant tumors were found, and no tumors occurred in treated animals in statistically significant incidences when compared with control animals.

In mice, a variety of neoplasms were observed in both treated and control animals. These neoplasms are not uncommon in this strain of mouse and none occurred in treated animals in statistically significant incidences when compared with control animals.

It is concluded that under the conditions of this bioassay, anthranilic acid was not carcinogenic for either Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 2-aminobenzoic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-37 Bioassay of Toxaphene for Possible Carcinogenicity (CAS No. 8001-35-2)

Toxaphene is an organochlorine insecticide that belongs to the class of compounds known as polychlorinated bicyclic terpenes with chlorinated camphenes predominating; an insecticide marketed as Strobane-T® (Tenneco Chemical Co., Piscataway, N.J.) is identical with toxaphene.

A bioassay of technical-grade toxaphene for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex were administered toxaphene at one of two doses for 80 weeks, then observed for 28 or 30 weeks. Time-weighted average doses for males were 556 or 1,112 ppm; for females they were 540 or 1,080 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups for toxaphene combined with 45 untreated male and 45 untreated female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 108-110 weeks.

Groups of 50 mice of each sex were administered toxaphene at one of two doses for 80 weeks, then observed for 10 or 11 weeks. Time-weighted average doses were 99 or 198 ppm for both males and females. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups for toxaphene combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-91 weeks.

Mean body weights attained by low- and high-dose female rats and high-dose male mice were lower than those of matched controls, but weights of other dosed groups were essentially unaffected by the toxaphene. Other clinical signs of toxicity in rats included generalized body tremors at week 53 in high-dose male and female animals, and later, leg paralysis, ataxia, epistaxis, hematuria, and vaginal bleeding, predominantly in the dosed groups of rats of each sex. Abdominal distention, diarrhea, dyspnea, and rough hair coats were common to both dosed rats and dosed mice. There were dose-related decreases in survival rates in mice but not in rats. Sufficient numbers of both rats and mice were at risk for the development of late-appearing tumors.

In the male rats, the incidence of follicular-cell carcinomas or adenomas of the thyroid was dose related ($P = 0.007$) using the pooled controls (matched controls 1/7, pooled controls 2/44, low-dose 7/41, high-dose 9/35). In the females, the incidence of follicular-cell adenomas of the thyroid was dose related using either the matched ($P = 0.022$) or pooled ($P = 0.008$) controls (matched controls 0/6, pooled controls 1/46, low-dose 1/43, high-dose 7/42). Direct comparisons of dosed and pooled-control groups but not matched controls showed significantly increased incidences of follicular-cell carcinomas or adenomas in the high-dose males ($P = 0.008$) and of follicular-cell adenomas in the high-dose females ($P = 0.021$). Two follicular-cell tumors in the high-dose males were carcinomas; all other follicular-cell tumors in the rats were adenomas.

In the mice, the incidence of hepatocellular carcinomas was dose related ($P < 0.001$) for both males (matched controls 0/10, pooled controls 4/48, low-dose 34/49, high-dose 45/46) and females (matched controls 0/9, pooled controls 0/48, low-dose 5/49, high-dose 34/49), using either matched or pooled controls. Direct comparisons showed that the incidences of hepatocellular carcinomas in low- and high-dose male mice and high-dose female mice were all significantly higher ($P < 0.001$) than those in the respective matched or pooled controls. Statistical significance was maintained when the incidence of hepatocellular carcinomas was combined with that of neoplastic nodules of the liver.

It is concluded that under the conditions of this bioassay, toxaphene was carcinogenic in male and female B6C3F$_1$ mice, causing increased incidences of hepatocellular carcinomas. The test results also suggest carcinogenicity of toxaphene for the thyroid of male and female Osborne-Mendel rats.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Positive

TR-38 Bioassay of Aroclor® for Possible Carcinogenicity (CAS No. 27323-18-8)*

Aroclor® is the registered trademark of the Monsanto Chemical Company for their polychlorinated biphenyls (PCBs). PCBs were developed in 1929 primarily for use as heat transfer fluids and dielectrics (insulators). Aroclor® 1254, a biphenyl containing approximately 54% chlorine, is a nonflammable heat transfer agent which functions in the range of 250-360° C.

A bioassay of Aroclor® 1254 for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered Aroclor® 1254 at one of three doses, either 25, 50, or 100 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks.

Mean body weights of males and females receiving mid and high doses and females receiving low doses of the chemical were consistently below those of the correspond-

ing controls, beginning at about week 10 of the study. The decrease in survival among males, but not among females, showed a significant dose-related trend. Adequate numbers of animals of both sexes survived for meaningful statistical analyses of the incidences of tumors.

The combined incidences of lymphomas and leukemias showed a significant dose-related trend in males (controls 3/24, low-dose 2/24, mid-dose 5/24, high-dose 9/24, P = 0.009). However, the direct comparisons of each dosed group with those of the matched controls were not statistically significant, and the tumors cannot clearly be related to administration of with Aroclor® 1254.

Hepatocellular adenomas and carcinomas were found in the dosed groups, but not in the controls (males: mid-dose 1/24, high-dose 3/24; females: mid-dose 1/24, high-dose 2/24). Additionally, a high incidence of nonneoplastic hyperplastic nodules was noted in the dosed animals (males: controls 0/24, low-dose 5/24, mid-dose 8/24, high-dose 12/24; females: controls 0/23, low-dose 6/24, mid-dose 9/22, high-dose 17/24). Although the incidences of tumors were not significant, the occurrence of the hyperplastic nodules appeared to be related to administration of the chemical.

In the stomach, jejunum, or cecum, adenocarcinomas were observed in two dosed males and in two dosed females as well as a carcinoma in one dosed male. None of these lesions was found in control animals in this study. Historical incidences of these tumors at this laboratory (6/600 males [1%], 2/600 females [0.3%] suggest that the lesions, although not statistically significant, may be related to the administration of Aroclor® 1254.

It is concluded that under the conditions of this bioassay, Aroclor® 1254 was not carcinogenic in Fischer 344 rats; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to administration of the chemical. In addition, the carcinomas of the gastrointestinal tract may be associated with administration of Aroclor® 1254 in both males and females.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal

*The technical report states that Aroclor® (CAS No. 11097-69-1) was the actual chemical. The CAS number listed in the technical report is for Chloro-1,1'-Biphenyl, the generic form of the compound tested; therefore, the CAS number for Aroclor® is used to track this study in the NTP CHEMTRACK database.

TR-39 Bioassay of Lasiocarpine for Possible Carcinogenicity (CAS No. 303-34-4)

Lasiocarpine is a pyrrolizidine alkaloid that is found in the seeds of *Heliotropium lasiocarpum*, *Heliotropium europaeum*, and several other plant species, all members of the family *Boraginaceae*.

A bioassay of lasiocarpine for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats.

Groups of 24 rats of each sex were administered lasiocarpine at one of three doses, either 7, 15, or 30 ppm, for 104 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104 weeks.

Mean body weights of the high-dose male and female rats were lower than those of the matched-control groups throughout most of the study, while weights of the mid-dose rats were lower only during the second year, and weights of the low-dose groups were unaffected. There was a positive dose-related trend in mortality for both sexes, with none of the high-dose animals, only five of the mid-dose animals, 23 of the low-dose animals, and 43 of the matched controls surviving to termination of the study. In spite of these early deaths, all male rats except one low-dose animal and one high-dose animal developed tumors, and among the females, 23 low-dose and 22 mid-dose animals developed tumors. Time-adjusted analysis of the incidence of tumors was performed in the female rats.

In male rats, there was a positive dose-related trend (P<0.001) in the incidence of angiosarcoma of the liver; furthermore, the incidences in the mid- and high-dose groups, but not that in the low-dose, were significantly higher (P<0.001, both groups) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 11/24, high-dose 13/24). In females, the incidences in both the low- and mid-dose groups, but not that in the high-dose, were significantly higher (P = 0.002 and P = 0.005, respectively) than that in the controls (controls 0/24, low-dose 8/24, mid- dose 7/24, high-dose 2/9). Metastatic angiosarcomas were present in the lungs from a few of the rats in all three treated groups of both sexes.

In both male and female rats, there was a positive dose-related trend in the combined incidence of hepatocellular carcinoma and adenoma of the liver (males, P = 0.003; females, P<0.001); furthermore, the combined incidence of these tumors in the high-dose females, but not those in the low- and mid-dose, was significantly higher (P<0.001) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 1/24, high-dose 7/9). The P-value of the combined incidence in the high-dose males (P = 0.025) is above the 0.016 level required by the Bonferroni inequality criterion, when multiple comparison is considered (controls 0/24, low-dose 0/24, mid-dose 3/24, high-dose 5/24). Nodular hyperplasia was observed in additional animals of each treated group of each sex. Thus, lasiocarpine was associated with proliferative lesions of hepatocytes as well as with angiosarcomas arising from endothelial cells of the liver.

The combined incidence of lymphoma or leukemia was significant in both the low- and mid-dose female groups (P≤ 0.018), but not in the high-dose group, perhaps because of the early deaths in this group (controls 2/24, low-dose 9/24, mid-dose 11/24, high-dose 1/23). The incidences of these tumors in the males were not significant.

It is concluded that under the conditions of this bioassay, lasiocarpine was carcinogenic in Fischer 344 rats producing hepatocellular tumors and angiosarcomas of the liver in both sexes and hematopoietic tumors in female animals.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive

TR-40 Bioassay of Hexachlorophene for Possible Carcinogenicity (CAS No. 70-30-4)

Hexachlorophene is a chlorinated bisphenol which was widely used as an antiseptic prior to 1972. It is highly effective against gram-positive bacteria and many pathogenic fungi.

A bioassay of hexachlorophene for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered hexachlorophene at one of three doses, either 17, 50, or 150 ppm, for 105-106 weeks. Higher doses of 200-600 ppm, used in 8-week subchronic studies, induced neuronal necrosis of the brain and clinical signs of toxicity. Matched-control groups consisted of 24 untreated rats of each sex. All surviving animals were killed at 105-106 weeks.

Mean body weights of the rats were unaffected by the hexachlorophene, and no clinical signs of toxicity were recorded. Survival also was unaffected, and adequate numbers of animals survived, permitting meaningful evaluation of the incidences of late-appearing tumors.

No tumors were present in a statistically significant incidence at any site in the treated rats.

It is concluded that under the conditions of this bioassay, hexachlorophene did not induce malignant or benign tumors in Fischer 344 rats.

Synonym: 2,2'-methylenebis(3,4,6-trichlorophenol)

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative

TR-41 Bioassay of Chlorothalonil for Possible Carcinogenicity (CAS No. 1897-45-6)

Chlorothalonil is a broad-spectrum fungicide which has been in use in the United States since 1963. It is registered for foliar and root applications on vegetables, fruits, green house plants, and turf, and as a seed treatment for cotton. Chlorothalonil is also used in formulating paints and stains for mildew resistance.

A bioassay of technical-grade chlorothalonil for possible carcinogenicity was conducted by administering the test chemical in the diet to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered chlorothalonil at one of two doses for 80 weeks, then observed for 30-31 weeks. Time-weighted average doses for both males and females were 5,063 or 10,126 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 55 untreated male or female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 110-111 weeks.

Groups of 50 mice of each sex were administered chlorothalonil at one of two doses for 80 weeks, then observed for 11-12 weeks. Time-weighted average doses for males were 2,688 or 5,375 ppm, and for females, 3,000 or 6,000 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male or female mice from similar bioassays of five other test chemicals. All surviving mice were killed at 91-92 weeks.

Clinical signs that appeared with increasing frequency in dosed rats included hematuria and, from week 72 until termination of the study, bright-yellow urine. Since the dosed female mice did not have depression in mean body weights or decreased survival compared with the controls, they may have been able to tolerate a higher dose.

In rats, adenomas and carcinomas of the renal tubular epithelium occurred with a significant dose-related trend in both the males ($P = 0.030$) and the females ($P = 0.007$). These neoplasms also occurred at a higher incidence in the high-dose males ($P = 0.035$) and the high-dose females ($P = 0.016$) than in the corresponding controls (males: pooled controls 0/62, low-dose 3/46, high-dose 4/49; females: pooled controls 0/62, low-dose 1/48, high-dose 5/50). These tumors included both adenomas and carcinomas which are considered to be histogenically related. Thus these findings are interpreted as sufficient evidence for the carcinogenicity of chlorothalonil.

In mice, no tumors were found to occur at a greater incidence among dosed animals than among controls.

It is concluded that under the conditions of this bioassay, technical-grade chlorothalonil was carcinogenic to Osborne-Mendel rats, producing tumors of the kidney. Chlorothalonil was not carcinogenic for $B6C3F_1$ mice.

Synonym: 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-42 Bioassay of 5-Azacytidine for Possible Carcinogenicity (CAS No. 320-67-2)

5-Azacytidine, a synthetic analogue of cytidine, has been used as an investigational anticancer drug in the United States since 1970.

A bioassay of 5-azacytidine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered 5-azacytidine at one of two doses, either 2.6 or 5.2 mg/kg body weight, in buffered saline three times per week for 34 weeks, and were then observed for 46 or 47 weeks. Controls consisted of groups of 15 rats of each sex that received injections of buffered saline (vehicle controls) and 15 rats of each sex that were untreated (untreated controls). All surviving rats were killed at 80 or 81 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 2.2 or 4.4 mg/kg body weight, in buffered saline three times per week for 52 weeks, and were then observed for 29 or 30 weeks. Controls consisted of groups of 15 mice of each sex that received injections of buffered saline (vehicle controls) and 15 mice of each sex that were untreated (untreated controls). All surviving mice were killed at 81 or 82 weeks.

5-Azacytidine was toxic to the animals in this bioassay, since mean body weights of both treated rats and treated mice were lower than those of the corresponding vehicle controls, and since none of the high-dose male and female rats and high-dose female mice lived to the end of the bioassay. In treated male and female rats and male mice, survival was inadequate for meaningful statistical analyses of the incidences of tumors.

Only one male and three female high-dose rats had tumors, and none of the tumors in the low-dose group of either sex were present at a significantly increased incidence using any of the statistical tests. Bone-marrow atrophy was present in both treated groups of both sexes of rats.

Only five high-dose male mice and one high-dose female mouse had neoplasms. In low-dose female mice, however, lymphocytic and granulocytic neoplasms of the hematopoietic system occurred in 17 animals, even though only 54% survived until week 81. Granulocytic neoplasms were observed in 10/29 low-dose female mice, but in no other group, and were significant ($P = 0.010$) compared with the vehicle controls. The incidence of combined lymphoma and granulocytic neoplasms was highly significant in the low-dose females (vehicle controls 0/14, low-dose 17/29, $P < 0.001$). No tumors were observed at a significant incidence in male mice. Bone-marrow atrophy was present in high-dose female mice.

It is concluded that under the conditions of this bioassay, the short life span and short duration of treatment of Sprague-Dawley rats of either sex and of male $B6C3F_1$ mice precluded evaluation of the carcinogenicity of 5-azacytidine in these groups; however, the induction of tumors of the hematopoietic system in female $B6C3F_1$ mice was associated with the administration of 5-azacytidine.

Synonym: 4-amino-1-β-D-ribofuranosyl-1,3,5-triazine-2(1H)-one

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Inadequate Study
Female Mice:	Positive

TR-43 Bioassay of Emetine for Possible Carcinogenicity (CAS No. 483-18-1)*

A bioassay of emetine, an amebicide and anticancer drug, for possible carcinogenicity was conducted by administering the test material by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered emetine at one of two doses, either 0.5 or 1 mg/kg body weight, three times per week for 52 weeks, and then observed for an additional 31 or 32 weeks. Control groups of each sex consisted of 10 untreated rats (untreated controls) and 10 rats injected with buffered saline (vehicle controls). Pooled-control groups, used for statistical evaluation, consisted of the vehicle-control rats of each sex for this study combined with 15 vehicle-control rats of each sex from a similar bioassay of another test chemical. All surviving rats were killed at 83 or 84 weeks.

Initially, groups of 35 mice of each sex were administered emetine at one of two doses, either 3.2 or 6.4 mg/kg body weight (mid- and high-dose), three times per week. Control groups of each sex consisted of 15 untreated mice (untreated controls) and 15 mice injected with buffered saline (vehicle controls). Due to high mortality rates in the initial treated groups, additional groups of 35 mice of each sex were later put on study at 1.6 mg/kg (low-dose), together with 10 untreated-control and 10 vehicle-control mice of each sex. The high-dose males were treated for 28 weeks and the mid- and high-dose females for 40 and 33 weeks, respectively. Mid- and low-dose male mice and low-dose female mice were treated for 52 weeks, and then observed for an additional 20 or 26 weeks. All surviving mice were killed at 78-83 weeks.

Emetine was toxic to male rats at the high dose, to both sexes of mice at the high and mid doses and to a lesser extent at the low dose, as shown by the low survival in these groups. Twenty-six percent of the high-dose male rats and 69% of the high-dose female rats, but none of the high- and mid-dose mice of either sex, survived to the end of the study. In the low-dose mice, 30/35 males and 21/35 females lived at least 1 year, and the median time on study was 72 weeks for males and 59 weeks for females.

No tumors occurred at a statistically significant incidence in treated rats or mice compared with controls; however, it should be noted that in this study, treatment of both species was stopped at week 52 and the studies were terminated by week 83, which is earlier than in current bioassays where animals are treated until termination of the studies at 2 years. In addition, there was poor survival among the treated mice.

It is concluded that the results of this study do not allow evaluation of the possible carcinogenicity of emetine.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

*The technical report states that Emetine Hydrochloride (CAS No. 316-42-7) was the actual chemical tested rather than Emetine in its pure form; therefore, the CAS number for Emetine Hydrochloride is used to track this study in the NTP CHEMTRACK database.

TR-44 In Vitro Carcinogenesis: Guide to the Literature, Recent Advances and Laboratory Procedures

Note to the Reader: This document is the published proceedings of a seminar and workshop held in July, 1976 and sponsored by the National Cancer Institute.

Report Date: 1978

TR-45 Bioassay of Chlorpropamide for Possible Carcinogenicity (CAS No. 94-20-2)

Chlorpropamide is an oral hypoglycemic agent of the arylsulfonylurea type. Chlorpropamide was selected for testing in the carcinogenesis program because it is used extensively and for prolonged periods in humans.

A bioassay of chlorpropamide for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered chlorpropamide as follows: rats 5 days per week for 103 to 105 weeks at 3,000 or 6,000 ppm, and mice 5 days per week for 34 weeks at 5,000 or 10,000 ppm, followed by 70 weeks at 2,500 or 5,000 ppm. The time-weighted average doses for mice were 3,317 ppm for low-dose males and females, and 6,635 ppm for high-dose males and females. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. All surviving rats and mice were killed at 103 to 105 weeks.

Mean body weights of both low- and high-dose rats were lower than those of the matched controls throughout the study. In mice, doses were reduced at week 34, due to early deaths in the high-dose groups; following this adjustment the treated mice gained weight, but the weights never reached those of the controls. Survival of the treated rats and the low-dose mice was adequate for meaningful statistical analyses of the incidences of tumors.

In both rats and mice, the incidences of tumors among the treated groups were not significantly increased in comparison with matched controls.

It is concluded that under the conditions of this bioassay, chlorpropamide was not carcinogenic for Fischer 344 rats or $B6C3F_1$ mice.

Synonym: 1-[(p-chlorophenyl)sulfonyl]-3-propylurea

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-46 Bioassay of Ethionamide for Possible Carcinogenicity (CAS No. 536-33-4)

A bioassay of the chemotherapeutic drug ethionamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 34 or 35 mice of each sex were administered ethionamide at one of the following doses, either 1,500 or 3,000 ppm for the rats and either 1,000 or 2,000 ppm for the mice. The animals were treated 5 days per week for 78 weeks, then observed for an additional 25 or 26 weeks. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. All surviving animals were killed at 103 or 104 weeks.

Mean body weights of the treated rats and mice were lower than those of the corresponding matched controls during most or all of the study. Survival in the rats was sufficient to allow development of late-appearing tumors. In the mice, survival of the high-dose males (27%), matched-control males (7%), and low-dose females (37%) to the end of the study was low, and the deaths were associated with suppurative lung lesions. However, tests for dose-related trend in mortality were not significant in either sex, and 47% or more of all groups of mice except control males were alive at 78 weeks.

In the rats, a variety of neoplasms were observed in treated and control groups of each sex. The lesions were of types commonly found in Fischer 344 rats, and none of the incidences of tumors in dosed animals were statistically significant when compared with controls.

In the mice, the incidences of malignant lymphoma were slightly higher in dosed than in control mice (males: controls 2/15, low-dose 8/34, high-dose 4/34; females: controls 2/15, low-dose 4/31, high-dose 10/34). The incidences were not significant by any of the statistical tests used, including the Tarone and Cox tests using the life-table method.

It is concluded that under the conditions of this bioassay, ethionamide was not carcinogenic in either Fischer 344 rats or $B6C3F_1$ mice.

Synonym: 2-ethylthioisonicotinamide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-47 Bioassay of 4,4'-Thiodianiline for Possible Carcinogenicity (CAS No. 139-65-1)

4,4'-Thiodianiline is an intermediate in the manufacture of several diazo dyes.

A bioassay of 4,4'-thiodianiline for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered 4,4'-thiodianiline 5 days per week at one of the following doses, either 1,500 or 3,000 ppm for the rats and either 2,500 or 5,000 ppm for the mice. The period of administration of the chemical was 68-72 weeks for the rats and 77 or 79 weeks for the mice, depending on the length of survival time of the animals. Matched controls consisted of groups of 15 untreated rats and 14 untreated mice of each sex. All surviving matched-control rats were killed at 104 weeks; all surviving matched-control mice were killed at 91 weeks.

The administration of 4,4'-thiodianiline resulted in marked reduction in mean body weights of the rats and mice of each sex, and all dosed animals died prior to the scheduled end of the study.

Tumors of epithelial origin were found in many organs, and all dosed rats except one were affected at one or more sites (males: skin, ear canal, lungs, liver, colon, and thyroid; females: ear canal, lung, liver, thyroid, and uterus). These tumors were not found among any of the matched-control animals.

In male rats, several of these neoplastic lesions occurred with statistically significant incidences in one or both of the dosed groups. The incidences of hepatocellular carcinoma (controls 0/15, low-dose 21/33, high-dose 10/33) and of follicular-cell carcinoma of the thyroid (controls 0/15, low-dose 28/33, high-dose 32/33) were significant in each of the groups at $P \leq 0.014$. The combined incidences of squamous-cell carcinoma and squamous-cell papilloma of the ear canal in the low- and high-dose groups of males were both significantly higher (low-dose $P = 0.001$, high-dose $P = 0.037$) than that in the control group (controls 0/15, low-dose 15/33, high dose 8/33). The first such tumor in the high-dose group was observed at 25 weeks.

Also in low-dose male rats, squamous-cell papilloma of the skin occurred in 4/33 animals, and squamous-cell carcinoma of the skin in 1/33, but no tumors of either type occurred in the controls. The incidences of these lesions were too low to have statistical significance. The majority of the squamous-cell tumors of the skin were located in one area near the commissure of the mouth. Only one such tumor occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the skin may be associated with administration of the chemical. Adenocarcinoma of the colon occurred in six low-dose male rats and in one high-dose male rat, but not in any of the controls. This incidence is not statistically significant; however, no such tumors occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the colon are considered to be related to administration of 4,4'-thiodianiline.

In female rats, the incidences of hepatocellular adenoma or carcinoma in the dosed groups were greater than those in the controls, but not statistically significant (controls 0/15, low-dose 6/32, high-dose 3/33). Follicular-cell carcinoma of the thyroid and adenocarcinoma of the uterus occurred in the females administered the test chemical at statistically significant incidences ($P < 0.001$) in both dosed groups (follicular-cell carcinoma: controls 0/14, low-dose 24/33, high-dose 32/32; adenocarcinoma: controls 0/15, low-dose 31/33, high-dose 23/32). Squamous-cell papilloma or carcinoma of the ear canal occurred at increased, but not statistically significant, incidences in female rats (controls 0/15, low-dose 6/33, high-dose 3/33). However, no such tumors occurred among the 235 historical-control female rats at this laboratory; thus, the tumors of the ear canal are considered to be related to administration of the chemical.

In mice of each sex, the incidence of hepatocellular carcinoma was statistically significant ($P < 0.001$) in each of the dosed groups (males: controls 1/13, low-dose 32/34, high-dose 22/24, females: controls 0/12, low-dose 32/34, high-dose 30/31). In the males, follicular-cell carcinoma of the thyroid occurred at statistically significant incidences ($P \leq 0.001$) in both the low- and high-dose groups (controls 0/14, low-dose 15/33, high-dose 20/23). In the females, the incidence was significant ($P = 0.002$) only at the high dose (controls 0/11, high-dose 15/30); however, when follicular-cell adenoma and carcinoma were combined, the incidences in both the low- and high-dose groups of females were significantly higher (low-dose $P = 0.025$, high-dose $P < 0.001$) than that in the control group (controls 0/11, low-dose 11/33, high-dose 18/30).

It is concluded that under the conditions of this bioassay, 4,4'-thiodianiline was carcinogenic for Fischer 344 rats, inducing tumors in the liver, thyroid, colon, and ear canal of male rats, and the thyroid, uterus, and ear canal of female rats. 4,4'-Thiodianiline was carcinogenic for $B6C3F_1$ mice, inducing tumors in the liver and thyroid of both males and females.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-48 Bioassay of Pyrazinamide for Possible Carcinogenicity (CAS No. 98-96-4)

A bioassay of the tuberculostatic drug pyrazinamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered pyrazinamide at one of two doses, either 5,000 or 10,000 ppm, for 78 weeks, and then observed for an additional 26 or 27 weeks. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. High-dose male mice died or were killed by week 92; all other surviving animals were killed at weeks 104 or 105.

Mean body weights of the dosed male rats were slightly lower than those of the matched controls, while mean body weights of the dosed females were more nearly comparable to those of the controls. A sufficient number of rats in each group was at risk to termination of the study at weeks 104-105 for the development of late-appearing tumors.

In mice, administration of pyrazinamide had no consistent effect on mean body weights. Survival to termination of the study was low, particularly among the control groups.

In rats, no lesions could clearly be related to administration of the chemical.

In mice, interstitial and suppurative myocarditis in the dosed animals and suppurative bronchopneumonias in both dosed and matched control mice of each sex were associated with increased deaths. In the females, there was a significant positive dose-related trend ($P = 0.037$) in the incidence of lymphoma (matched controls 0/13, low-dose 2/25, high-dose 6/29); however, the incidences in each of the dosed groups were not significant when compared with that in the matched controls. In addition, the poor survival and the small size of the control group precluded making a clear association of the incidence of these tumors with administration of the chemical.

It is concluded that under the conditions of this bioassay, the early deaths and small size of the control group precluded a conclusion regarding the carcinogenicity of pyrazinamide in female $B6C3F_1$ mice. Pyrazinamide was not carcinogenic for Fischer 344 rats or for male mice.

Synonym: pyrazinecarboxamide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Inadequate Study

TR-49 Bioassay of Acronycine for Possible Carcinogenicity (CAS No. 7008-42-6)

Acronycine, an alkaloid derived from the bark of the Australian scrub ash, has been investigated as an experimental anticancer drug. Acronycine was selected for screening in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that may be used for prolonged periods in humans.

A bioassay of acronycine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Initially, groups of 35 rats of each sex were administered acronycine at one of two doses, either 7.5 or 15 mg/kg body weight, in a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated rats (untreated controls) and 10 rats injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, new dosed groups of 35 rats of each sex were started later at a dose of 3.75 mg/kg. Additional groups of 10 untreated and 10 vehicle controls of each sex were also started. The rats were administered the acronycine or the vehicle for 51 or 52 weeks, then observed for an additional 28-30 weeks. All surviving rats were killed at 80-82 weeks.

Initially, groups of 35 mice of each sex were administered acronycine at one of two doses, either 12.5 or 25 mg/kg body weight, in a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated mice (untreated controls) and 10 mice injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, two additional dosed groups were started later: 35 mice of each sex at 6 mg/kg and 40 mice of each sex at 2 mg/kg, together with 10 untreated controls and 10 vehicle controls of each sex for the groups dosed at 6 mg/kg, and 20 untreated controls and 20 vehicle controls for the groups dosed at 2 mg/kg. Periods of administration of the chemical to the mice varied from 25 weeks to 92 weeks, depending on toxicity or length of time of survival. Surviving control animals were killed at 78-105 weeks.

Acronycine was toxic to rats and mice of each sex at the doses used in this bioassay, as shown by the high mortality rates in all but the low-dose groups and by the lower mean body weights in dosed rats and mice at all doses throughout most of the bioassay.

Because of this high number of deaths, time-adjusted statistics are used for the analyses of all incidences of tumors.In male rats, the dose-related trend in the mid- and high-dose groups for the incidence of osteosarcoma at all sites was significant ($P = 0.002$) using the respective vehicle-control group (vehicle controls 0/8, mid-dose 13/30, high-dose 12/18). Comparisons of the individual groups with respective control groups were also significant for the mid-dose ($P = 0.022$) and high-dose

(P = 0.002) groups, but not for the low-dose group. In female rats, osteosarcoma was observed only in 1/8 high-dose animals.

Sarcomas and other related tumors of the peritoneum were observed in all three dosed groups of both male and female rats, but in none of the control groups (males: low-dose 5/30, mid-dose 3/26, high-dose 7/16; females: low-dose 1/35, mid-dose 5/30, high-dose 13/28). In both sexes, the dose-related trends were significant (males, P = 0.006; females, P = 0.002), and the comparison of the incidences in the high-dose females with the vehicle-control group was significant (P = 0.016). None of the incidences in the individual dosed groups of males were significant when compared with vehicle controls. However, since the tumors were observed in all dosed groups but did not occur in historical-control animals at this laboratory, they are considered to be related to the administration of the chemical.

In female rats, the incidence of all tumors of epithelial origin of the mammary gland was significant only at the low dose (low-dose vehicle controls 1/10, low-dose 22/35, P = 0.004). Adenocarcinomas of the mammary gland were observed in seven low-dose, five mid-dose, and two high-dose female rats, but in no control females. The reverse dose relationship of both benign and malignant tumors was probably due to the higher number of early deaths which occurred in the high-dose group.

In mice, the low survival in all dosed groups except the low-dose animals precluded an evaluation of the significance of the incidences of tumors. Lymphomas occurred in low-dose groups of both males and females; however, the incidence of lymphoma in different control groups was highly variable. The high incidence in the low-dose vehicle controls may have been due to a procedural problem associated with the possibility of transfer of tumor cells or oncogenic viruses during the intraperitoneal injection of the test chemical.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and control mice and the possible procedural problems associated with the intraperitoneal injection of the chemical did not allow a determination to be made of the carcinogenicity of acronycine in this species.

In Sprague-Dawley rats, acronycine in the vehicle of 0.05% polysorbate 80 in phosphate-buffered saline was carcinogenic, producing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other related tumors of the peritoneum in both males and females.

Synonym: 3,12-dihydro-6-methoxy-3,3,12-trimethyl-7H-pyrano(2,3-c)acridin-7-one

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

TR-50 Bioassay of Acetohexamide for Possible Carcinogenicity (CAS No. 968-81-0)

Acetohexamide is an oral hypoglycemic agent of the arylsulfonyl-urea group with a potency between that of tolbutamide and chlorpropamide.

A bioassay of acetohexamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 35 rats of each sex were administered acetohexamide in the diet at one of two doses, either 10,000 or 20,000 ppm, for 103 weeks and then observed for 2 to 4 additional weeks. Matched controls consisted of 15 untreated rats of each sex. All surviving rats were killed at 105 to 107 weeks.

Groups of 35 mice of each sex were administered acetohexamide at one of two doses for 103 weeks and then observed for 4 or 5 additional weeks. Time-weighted average doses were 6,359 or 12,718 ppm. Matched controls consisted of 15 untreated mice of each sex. All surviving mice were killed at 107 or 108 weeks.

Mean body weights of the dosed rats and mice of both sexes were lower than those of the corresponding matched controls throughout the study, and the depressions in weight were dose related. Except for the female mice, sufficient numbers of animals survived long enough to be at risk for development of late-appearing tumors.

In the rats, the only tumor occurring with greater incidence in dosed than in matched-control animals was leukemia (males: matched controls 0/15, low-dose 10/35, high-dose 4/35; females: matched controls 0/14, low-dose 7/35, high-dose 4/34). Only the incidence in the low-dose males was statistically significant (P = 0.018). All of these animals had undifferentiated (mononuclear cell) leukemia, which commonly occurs spontaneously in Fischer 344 rats, except for two with lymphocytic leukemia. The incidence of combined leukemia and lymphoma in historical-control male rats at this laboratory in the bioassay program to date is 24/235 (10.2%), which is higher than that in the matched controls. Thus, the incidence in the low-dose males cannot be clearly associated with administration of the test chemical.

In the mice, the only neoplasms that occurred at a higher incidence in dosed groups than in matched controls were lymphomas in the males, but the incidences were not statistically significant (matched controls 1/15, low-dose 9/35, high-dose 3/34). These types of lesions are found commonly in untreated B6C3F$_1$ mice. The incidence of lymphomas in the historical-control male mice is 28/536 (5.2%).

It is concluded that under the conditions of this bioassay, acetohexamide was not carcinogenic for either Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 1-[(p-acetylphenyl)-sulfonyl]-3-cyclohexyl-urea

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-51 Bioassay of Tolazamide for Possible Carcinogenicity (CAS No. 1156-19-0)

Tolzamide is an oral hypoglycemic agent of the arylsulfonylurea type, similar to tolbutamide, chlorpropamide, and acetohexamide.

A bioassay of the hypoglycemic drug tolazamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered tolazamide at one of two doses, either 5,000 or 10,000 ppm, for 103 weeks. Matched controls consisted of 15 rats and 15 mice of each sex. All surviving rats and mice were killed at 104 or 105 weeks.

Survival rates for the dosed rats of each sex were higher than those for the matched controls, and were adequate for the development of late-appearing tumors. Survival rates for the mice were lower than those for the rats, particularly for the dosed females (matched controls 67%, low-dose 34%, high-dose 32%). However, a large number of these deaths in the dosed females occurred after 90 weeks on study, and survival of both males and females was adequate for the development of late-appearing tumors.

All observed tumors were of types commonly found in the strains of animals used, and there were no statistically significant increases in the incidence of tumors in the dosed animals as compared with controls.

It is concluded that under the conditions of this bioassay, tolazamide was not carcinogenic for Fischer 344 rats or $B6C3F_1$ mice.

Synonym: N-(p-toluenesulfonyl)-N'-hexamethyleniminourea

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-52 Bioassay of 3-Nitropropionic Acid for Possible Carcinogenicity (CAS No. 504-88-1)

3-Nitropropionic acid was selected for testing for carcinogenic activity because it was known to demonstrate varying degrees of toxicity in man and animals, and because its use in food preparations and its identification as a contaminant in foods suggested there was a possibility of long-term human exposure.

A bioassay of 3-nitropropionic acid (95% pure) for possible carcinogenicity was conducted by administering the test chemical by gavage to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered 3-nitropropionic acid at one of the following doses by gavage 5 days per week. For male rats, the doses were 0.425 or 0.85 mg/animal/day; for females, they were 0.6 or 1.2 mg/animal/day. For both sexes of mice, the doses were 0.375 or 0.75 mg/animal/day. The rats were administered the chemical for 110 weeks and the mice for 104 weeks. The controls consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at 111 weeks and all surviving mice at 104 or 105 weeks.

Mean body weights and mortality of the dosed animals were not markedly affected by 3-nitropropionic acid under the conditions of this bioassay, indicating that the maximum tolerated dose may not have been reached. The various clinical signs observed were common to both dosed and control groups.

In rats, the combination of neoplastic nodule of the liver and hepatocellular carcinoma occurred in the males with a significant dose-related trend ($P = 0.010$) and with a higher incidence ($P = 0.012$) in the high-dose group of animals than in the controls (controls 0/49, low-dose 3/50, high-dose 6/49). All but one of these tumors were neoplastic nodules. In the females, only two neoplastic nodules occurred, one in each of the dosed groups. Biliary hyperplasia occurred at a higher incidence in the dosed males than in the corresponding controls (controls 19/50, low-dose 32/50, high-dose 36/50), but the incidence of this lesion in the dosed females was not increased as compared with controls. There was also a dose-related trend ($P = 0.033$) in the incidence of pancreatic islet-cell adenoma in the male rats (controls 4/49, low-dose 6/50, high-dose 11/50); however, direct comparisons of incidences in the dosed and control groups were not statistically significant. The historical incidence of pancreatic islet-cell adenoma among 100 control Fischer 344 rats at the laboratory was 7/100 (7%). In addition, focal myocardial fibrosis was observed at a higher incidence in dosed rats than among controls (males: controls 1/4, low-dose 17/49, high-dose 24/48; females: controls 2/48, low-dose 9/46, high-dose 9/50).

In mice, each type of neoplasm found in the dosed and control mice has been encountered previously as a spontaneous lesion. No specific tumor was found to occur at a statistically significantly higher incidence among dosed mice than among the respective control groups.

It is concluded that under the conditions of this bioassay, there was an elevated incidence of hepatocellular neoplasms, primarily benign, and of islet-cell adenomas of the pancreas in male Fischer 344 rats receiving 3-nitropropionic acid as compared with controls; however, there was no conclusive evidence that 3-nitropro-

pionic acid was carcinogenic in these animals. The chemical was not carcinogenic in female rats or in male or female B6C3F$_1$ mice.

Synonyms: β-nitropropionic acid; hiptagenic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-53 Bioassay of 2-Amino-5-Nitrothiazole for Possible Carcinogenicity (CAS No. 121-66-4)

2-Amino-5-nitrothiazole is an antiprotozoal drug for animals which is now used in the form of the acetyl derivative to control histomoniasis (blackhead) in turkeys. The use of acetyl-2-amino-5-nitrothiazole in animal feed and the allowable residues in food products from treated animals (0.1 ppm) are regulated by the Food and Drug Administration.

A bioassay of 2-amino-5-nitrothiazole for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were fed 2-amino-5-nitrothiazole at one of the following doses, either 300 or 600 ppm for rats, and either 50 or 100 ppm for mice. The rats were dosed for 110 weeks, followed by 1 week of observation; the mice were dosed for 104 weeks. Matched controls consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at week 111, all surviving mice at week 104.

The mean body weights of the groups of rats and mice fed 2-amino-5-nitrothiazole in the diet were slightly lower than those of the controls throughout most of the period of administration. No other clinical signs related to administration of the chemical were noted. There was a dose-related trend in mortality only in the male rats; however, sufficient numbers of rats were at risk in all groups for development of late-appearing tumors.

In male rats, there was a significant dose-related trend ($P = 0.044$) in the incidences of malignant lymphomas, lymphocytic leukemias, or undifferentiated leukemias, although the results of direct comparisons of incidences in each of the dosed groups with those in the controls were not significant. There was also a significant dose-related trend in the incidence of granulocytic leukemia in the male rats ($P = 0.014$) and a significantly increased incidence of this tumor ($P = 0.023$) in the high-dose group (matched controls 2/50, low-dose 4/50, high-dose 9/49). When the incidences of all neoplasms of the hematopoietic system lymphomas and all leukemias) were combined, greater significance was attained for both the dose-related trend ($P = 0.001$) and the direct comparison ($P = 0.002$) of the incidence of the high-dose group with that in the matched controls (controls 13/50, low-dose 9/50, high-dose 28/49). The reliability of the incidence of hematopoietic tumors in the male controls was supported by that for male controls observed in a similar bioassay of another test chemical at the same laboratory (13/50). The incidences of the combined hematopoietic tumors in the dosed female rats were not significant when compared with the incidence in the matched controls.

In female rats, there was a significant dose-related trend in the incidence of chromophobe adenomas of the pituitary ($P = 0.016$) and a higher incidence ($P = 0.021$) in the high-dose group than in the matched controls (controls 19/45, low-dose 29/47, high-dose 29/44). The incidence of this lesion in dosed male rats was much lower than that in dosed females, and the dose-related trend ($P = 0.048$) was only marginally significant (controls 3/46, low-dose 3/45, high-dose 8/43). The incidences of chromophobe adenomas of the pituitary which were observed in control groups of rats used in a similar bioassay of another test chemical at the same laboratory were 13/49 (27%) for the males and 26/50 (52%) for the females. Because of the variability in incidences of the tumor among different control groups, the occurrence of chromophobe adenomas of the pituitary in the dosed female rats cannot be clearly associated with the administration of 2-amino-5-nitrothiazole.

Also in female rats, there was a higher incidence of endometrial stromal polyps of the uterus in the low-dose group ($P = 0.023$) than in the matched controls (controls 2/50, low-dose 9/49, high-dose 3/50). Since, however, only three high-dose animals had this tumor, the occurrence of uterine tumors in the low-dose group cannot be clearly associated with administration of the test chemical.

In the mice, no neoplasms were observed at a statistically significant incidence in the dosed groups when compared with the controls.

It is concluded that under the conditions of this bioassay, the occurrence of tumors of the hematopoietic system, i.e., lymphoma and granulocytic leukemia, in dosed male Fischer 344 rats was associated with administration of 2-amino-5-nitrothiazole. 2-Amino-5-nitrothiazole was not carcinogenic in female Fischer 344 rats or in male or female B6C3F$_1$ mice.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-54 Bioassay of 2,4-Dinitrotoluene for Possible Carcinogenicity (CAS No. 121-14-2)

2,4-Dinitrotoluene, a precursor in the synthesis of azo dyes, was selected for bioassay by the National Cancer

Institute along with other dye intermediates in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry. 2,4-Dinitrotoluene is used by the munitions industry as a modifier for smokeless powders and, to a limited extent, as a gelatinizing and waterproofing agent in military and commercial explosive compositions.

A bioassay of practical-grade 2,4-dinitrotoluene for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. 2,4-Dinitrotoluene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For male and female rats, the high and low time-weighted average dietary concentrations of 2,4-dinitrotoluene were 0.02 and 0.008 percent, respectively. For male and female mice, the high and low time-weighted average concentrations were 0.04 and 0.008 percent, respectively. After a 78-week period of compound administration, observation of the rats continued for an additional 26 weeks and observation of the mice continued for 13 additional weeks.

For the chronic rat bioassay, 25 rats of each sex were placed on test as high dose controls, and 50 rats of each sex served as the low dose controls. For the mice, 50 males and 50 females were placed on test as controls for each of the high dose and low dose groups.

In both species the survival in all groups was adequate for statistical analysis of late-appearing tumors.

In the male rats, a significantly increased incidence of fibroma of the skin and subcutaneous tissue occurred in both the high and the low dose groups when compared to their respective controls. A statistically significant incidence of fibroadenoma of the mammary gland occurred in the high dose female rats.

Among the mice a variety of tumors was observed but none were considered to be associated with the dietary administration of 2,4-dinitrotoluene.

Under the conditions of this bioassay dietary administration of 2,4-dinitrotoluene to Fischer 344 rats induced benign tumors (i.e., fibroma of the skin and subcutaneous tissue in males and fibroadenoma of the mammary gland in females). No evidence was provided for the carcinogenicity of the compound in B6C3F$_1$ mice of either sex.

Synonyms: 1-methyl-2,4-dinitrobenzene; 2,4-dinitrotoluol; 2,4-DNT

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-55 Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity (CAS No. 107-06-2)

1,2-Dichloroethane, a chlorinated aliphatic hydrocarbon, is one of several halogenated solvents selected for bioassay by the National Cancer Institute. Although the major use of 1,2-dichloroethane is as an intermediate in the synthesis of vinyl chloride, the compound also finds application as a constituent in lead-containing antiknock preparations, as an ingredient in fumigant-insecticide formulations and, to a more limited extent, as a component of metal degreasing mixtures. 1,2-Dichloroethane is additionally employed as an intermediate in the synthesis of the chlorinated solvents 1,1,1-trichloroethane, trichloroethylene, and perchloroethylene and as a constituent of rubber cements and acrylic-type adhesive formulations.

A bioassay of technical-grade 1,2-dichloroethane for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. 1,2-Dichloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The 78-week period of chemical administration was followed by an observation period of 32 weeks for the low dose rats of both sexes. The last high dose male rat died after 23 weeks of observation and the last high dose female rat died after 15 weeks of observation. All treated groups of mice were observed for an additional 12 or 13 weeks following chemical administration.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average high and low doses of 1,2-dichloroethane in the chronic study were 95 and 47 mg/kg/day, respectively, for rats of both sexes. The high and low time-weighted average doses for the male mice were 195 and 97 mg/kg/day, respectively, and 299 and 149 mg/kg/day, respectively, for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same times that dosed animals were gavaged with the 1,2-dichloroethane mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

A statistically significant positive association between dosage and the incidence of squamous-cell carcinomas of the forestomach and hemangiosarcomas of the circulatory system occurred in the male rats, but not in the females. There was also a significantly increased incidence of adenocarcinomas of the mammary gland in female rats.

The incidences of mammary adenocarcinomas in female mice were statistically significant. There was a statistically significant positive association between chemical administration and the combined incidences of endometrial stromal polyps and endometrial stromal

sarcomas in female mice. The incidence of alveolar/bronchiolar adenomas in both male and female mice was also statistically significant.

Under the conditions of this study, 1,2-dichloroethane was carcinogenic to Osborne-Mendel rats, causing squamous-cell carcinomas of the forestomach, hemangiosarcomas, and subcutaneous fibromas in male rats and causing mammary adenocarcinomas in female rats. This compound was also found to be carcinogenic to $B6C3F_1$ mice, causing mammary adenocarcinomas and endometrial tumors in female mice, and causing alveolar/bronchiolar adenomas in mice of both sexes.

Synonyms: ethylene chloride; ethylene dichloride; alpha beta dichloroethane

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-56 Bioassay of N,N'-Dicyclohexylthiourea for Possible Carcinogenicity (1212-29-9)

N,N'-dicyclohexylthiourea is a chemical intermediate used in the production of dicyclohexylcarbodimide, a reagent used in the synthesis of peptide and phosphodiester internucleotide bonds.

A bioassay of N,N'-dicyclohexylthiourea for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered N,N'-dicyclohexylthiourea at one of two doses, either 25,000 or 50,000 ppm, for 109 weeks for rats or 104 weeks for mice. Matched controls consisted of 50 untreated rats or 50 untreated mice of each sex.

Mean body weights of male rats and male mice were unaffected by the compound, whereas mean body weights of the females of each species showed mild dose-related retardation over the bioassay period, when compared with the matched controls. Survival was sufficient to termination of the study in all groups of both rats and mice for the development of late-appearing tumors.

In male rats there was an increased incidence of hyperplasia of the follicular cells of the thyroid (males: controls 3/43, low-dose 16/49, high-dose 15/49; females: controls 1/48, low-dose 7/48, high-dose 5/49). The incidences of tumors of the follicular cells of the thyroid, although increased among the dosed male rats, were not statistically significant in either sex.

In mice, a variety of neoplasms of the type usually encountered in the $B6C3F_1$ strain were observed in both dosed and control animals. None of the tumors occurred at statistically significant incidences. Follicular-cell hyperplasia of the thyroid was observed at an increased incidence in both the dosed males and females (males: controls 3/39, low-dose 12/46, high-dose 9/45; females: controls 8/38, low-dose 22/46, high-dose 21/46).

An increase in proliferative lesions of the follicular cells of the thyroid was associated with the administration of N,N'-dicyclohexylthiourea in both Fischer 344 rats and $B6C3F_1$ mice. However, because statistical significance was not achieved and because thyroid tumors are not rare, spontaneous lesions in these strains of animals and occur with a variable incidence, it is concluded that under the conditions of this bioassay, N,N'-dicyclohexylthiourea was not demonstrated to be carcinogenic in either species.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-57 Bioassay of β-TGdR for Possible Carcinogenicity (CAS No. 789-61-7)

Beta-2'-deoxy-6-thioguanosine monohydrate (β-TGdR) is an experimental anticancer drug and a derivative of the anticancer drug 6-thioguanine (6-TG).

A bioassay of beta-2'-deoxy-6-thioguanosine monohydrate (β-TGdR) for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered β-TGdR in a buffered saline and polysorbate 80 vehicle at one of two doses, either 3.5 or 7 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 26 weeks. Controls consisted of groups of 10 rats of each sex, which were either administered the vehicle alone (matched vehicle controls) or were untreated (matched untreated controls). Pooled controls consisted of the matched vehicle controls of each sex from the current bioassay, combined with 20 corresponding vehicle controls of each sex from similar bioassays of two other test chemicals. All surviving rats were killed at 78 or 79 weeks.

Groups of 35 mice of each sex were administered the chemical in a buffered saline and polysorbate 80 vehicle at one of two doses, either 2 or 4 mg/kg, three times per week for 52 weeks, then observed for periods of up to 27 weeks, depending on length of survival. Because of severe toxicity at the high dose, resulting in loss of all mice by week 12 (males) or week 25 (females), additional groups of 35 mice of each sex were administered 1 mg/kg on the same schedule. Controls consisted of groups of 15 mice of each sex, which were either administered the vehicle or were untreated. Pooled controls consisted of groups of 15 vehicle-control animals of each sex from studies using the doses of 2 or 4 mg/kg, combined with

corresponding groups of 15 vehicle-control animals of each sex from the study using the dose of 1 mg/kg.

β-TGdR was toxic to rats at the doses used in this study. Mean body weights of the high- and low-dose rats of both sexes were lower than those of the corresponding vehicle controls throughout the study. There was also severe early mortality in the high-dose groups of both sexes and positive dose-related trends in mortality over the period of the bioassay. However, 66% of the low-dose males and 77% of the low-dose females survived until termination of the study.

In mice, β-TGdR was toxic at the doses originally selected. Mean body weights were not consistently affected; however, at the high dose only three males and seven females lived past week 7, and all were dead by week 25. In the mid-dose group, only 14% of the males and 6% of the females survived until termination of the study at week 79; in the low-dose group, the survival rate was 31% for the males and 29% for the females.

Because of the high mortality, time-adjusted statistical analyses were performed for both rats and mice.

In rats, the incidence of carcinomas of the ear canal (combined carcinomas and squamous-cell carcinomas) was statistically significant in both sexes. In males, the results of the test for dose-related trend were significant using either matched vehicle ($P = 0.046$) or pooled vehicle ($P = 0.014$) controls, but direct comparisons of dosed male rats with matched vehicle or pooled vehicle controls did not show significant differences (matched vehicle controls 0/10, pooled vehicle controls 0/28, low-dose 1/31, high-dose 2/7). In females, the results of the test for dose-related trend were significant using either matched vehicle ($P = 0.002$) or pooled vehicle ($P < 0.001$) controls, and the incidence in the high-dose group was significantly higher than that in either the matched vehicle ($P = 0.023$) or pooled vehicle ($P < 0.001$) controls (matched vehicle controls 0/9, pooled vehicle controls 0/28, low-dose 2/32, high-dose 6/13). There were no such ear canal tumors among 165 historical vehicle controls of either sex or among 220 female untreated controls at the laboratory, and only two such tumors occurred among 215 male untreated controls.

In mice, no tumors appeared in statistically significant incidences in the dosed groups compared with the matched vehicle controls, and there was no significant evidence of dose-related trend for any tumors. The incidences of the combination of lymphoma and leukemia were significantly higher in the matched vehicle controls of each sex than in the corresponding matched untreated controls (males: matched untreated controls 1/30, matched vehicle controls 19/29; females: matched untreated controls 2/30, matched vehicle controls 21/29). This high incidence in the matched vehicle controls may have been due to a systematic procedural problem associated with injection of the drug.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and vehicle-control groups of mice, as well as the possible procedural problem that may have affected the incidences of tumors in these groups, does not allow a determination to be made of the carcinogenic potential of β-TGdR in this species. β-TGdR in the vehicle of 0.05% polysorbate 80 was, however, carcinogenic in rats, producing carcinomas of the ear canal in the females and possibly also in the males.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equiocal
Female Rats:	Positive
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

TR-58 Bioassay of Thio-TEPA for Possible Carcinogenicity (CAS No. 52-24-4)

Thio-TEPA is an ethyleneimine alkylating agent that was introduced in 1953 for clinical use in cancer chemotherapy. At one time thio-TEPA was an important therapeutic drug in the management of ovarian carcinoma. It has been used effectively in the treatment of Hodgkins disease, bronchogenic carcinoma, bladder cancer, retinoblastoma, and breast cancer and for the control of pleural, pericardial, and peritoneal neoplastic effusions.

A bioassay of thio-TEPA for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 31-39 rats of each sex were administered thio-TEPA in phosphate-buffered saline at one of three doses, either 0.7, 1.4, or 2.8 mg/kg body weight, three times per week for a maximum of 52 weeks, then observed for additional periods of time. The maximum time on study (administration of chemical and observation) was 86 weeks. The groups at the low dose were started 69 weeks after those at the mid and high doses, because of high mortalities observed in the groups at the higher doses. Matched controls consisted of groups of 10 untreated rats and 10 vehicle-control rats of each sex. Pooled-control groups also were used. Surviving control rats were killed at 82-87 weeks; surviving dosed rats were killed at 81 or 82 weeks.

Groups of 35 mice of each sex were administered thio-TEPA at one of two doses, either 1.15 or 2.3 mg/kg body weight, three times per week for a maximum of 52 weeks, then observed for a maximum additional period of 34 weeks. Matched controls consisted of groups of 15 untreated mice and 15 vehicle-control mice of each sex. Pooled controls also were used. Surviving control and dosed mice were killed at 86 or 87 weeks.

Thio-TEPA was toxic to both rats and mice, causing decreased mean body weight gains and early deaths in the mid- and high-dose rats and in the high-dose mice. Because of the early deaths, statistical analyses were based only on time-adjusted incidences of tumors. Since all high-dose male and female rats had died by 21 weeks, microscopic evaluation of tissues was performed only on the low- and mid-dose animals.

In rats, the incidence of combined neoplasms of the hematopoietic system (lymphoma, lymphocytic leukemia, or granulocytic leukemia) was significant in the males in both the low-dose (P = 0.020) and mid-dose (P = 0.001) groups, using pooled controls (pooled controls 0/29, low-dose 6/34; pooled controls 0/30, mid-dose 6/16).

Squamous-cell carcinoma of the skin or ear canal occurred at a significant incidence in the male rats in both the low-dose (P = 0.009) and mid-dose (P = 0.023) groups, using pooled controls (pooled controls 0/29, low-dose 7/33; pooled controls 0/30, mid-dose 3/13) and in the mid-dose females (P < 0.001), using pooled controls (pooled controls 0/28, mid-dose 8/21); in addition, two low-dose females had such tumors, with none occurring in the corresponding low-dose controls.

The incidence of adenocarcinoma of the uterus was significant in the mid-dose female rats (P = 0.001), using pooled controls (pooled controls 0/28, mid-dose 7/21); in addition, two low-dose females had adenocarcinoma of the uterus, with no such tumor occurring in the corresponding low-dose controls.

In rats, neuroepitheliomas (neuroblastomas) or nasal carcinomas occurred in three low-dose males, two low-dose females, and two mid-dose females. Although these are not statistically significant incidences, these tumors did not occur among control animals and no such tumors have occurred in 380 Sprague-Dawley control rats of each sex in other bioassays at the same laboratory. Thus, they may be associated with administration of the chemical.

In the high-dose groups of both male and female mice, but not in the low-dose groups, the incidences of lymphoma or lymphocytic leukemia were significantly higher (P < 0.001) for each sex than those of either the vehicle or pooled controls (males: vehicle controls 1/8, pooled controls 1/18, low-dose 2/24, high-dose 26/28; females: vehicle controls 0/14, pooled controls 0/29, low-dose 5/26, high-dose 32/32).

In the low-dose male mice squamous-cell carcinoma was found in the skin of seven animals, in the preputial glands of six animals, and in the ear canal of two animals. A carcinoma of the preputial gland was also found in a high-dose male. When the incidences of the tumors at the different sites were combined, the incidence in the low-dose group was statistically significant using either the vehicle (P = 0.004) or the pooled (P < 0.001) controls (vehicle controls 0/8, pooled controls 0/18, low-dose 14/24, high-dose 1/2).

It is concluded that under the conditions of this bioassay, thio-TEPA was carcinogenic in both Sprague-Dawley rats and B6C3F$_1$ mice. In the rats, the chemical induced squamous-cell carcinoma of the skin or ear canal in both males and females, and hematopoietic neoplasms in the males; in the mice, it induced lymphoma or lymphocytic leukemia in both sexes and squamous-cell carcinoma in the skin and associated glands of males.

Synonym: tris(1-aziridinyl)phosphine sulfide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-59 Bioassay of Estradiol Mustard for Possible Carcinogenicity (CAS No. 22966-79-6)

A bioassay of the experimental anticancer drug estradiol mustard for possible carcinogenicity was conducted by administering the chemical by gavage to Sprague-Dawley rats and B6C3F$_1$ mice.

Groups of 35 rats and 34-36 mice of each sex were administered estradiol mustard at one of the following doses, either 0.62 or 1.25 mg/kg body weight for rats and either 15 or 30 mg/kg body weight for mice. The vehicle used for the test chemical consisted of 0.05% polysorbate 80 in phosphate-buffered saline. The rats and mice were dosed three times per week for 52 weeks, then observed for an additional 30-34 weeks. Controls consisted of groups of 10 rats and 15 mice of each sex that were not administered the chemical (untreated controls) and also of groups of 10 rats of each sex, 14 male mice, and 16 female mice administered the vehicle alone (vehicle controls). Pooled controls were also used. All surviving rats were killed at 84-86 weeks and all surviving mice at 82-86 weeks.

Mean body weights of male rats and male and female mice administered estradiol mustard were lower throughout the greater part of the study than those of corresponding vehicle or untreated controls; mean body weights of dosed female rats were unaffected. Administration of the test chemical had no significant effect on the survival of either male or female rats. A large number of dosed mice died prior to the end of the study. The numbers of dosed male mice which were at risk as long as 52 weeks were sufficient, however, for development of tumors appearing up to that time. Time-adjusted analysis and life-table analyses were applied to data obtained with the mice.

In rats, no tumors were observed in a statistically significant incidence in the animals administered estradiol mustard.

In mice, lymphoma or lymphocytic leukemia occurred at significant incidences in low-dose (P = 0.018) and high-dose (P < 0.001) groups of males compared with those in the pooled vehicle controls (controls 0/28, low-dose 6/32, high-dose 17/29) and at significant incidences in low-dose (P = 0.020) and high-dose (P = 0.002) groups of females compared with those in the corresponding vehicle controls (controls 0/14, low-dose 9/30, high-dose 11/23). In addition, the incidences of lymphoma were statistically significant for dose-related trend for both the males (P < 0.001) and the females (P = 0.003). Since lymphoma was observed in male mice as early as 25 weeks, life-table analyses of the incidence in each sex were performed.

The results indicated a dose association (P = 0.001) between the administration of estradiol mustard and the time of observation of lymphoma in either sex of mice.

In mice, alveolar/bronchiolar adenoma or carcinoma occurred at a significant incidence (P = 0.004) in the low-dose group of males compared with the pooled vehicle controls (controls 2/28, low-dose 12/30, high-dose 5/24) and at a significant incidence (P = 0.022) in the low-dose group of females compared with the pooled vehicle controls (controls 1/28, low-dose 7/27, high-dose 1/18). Sarcoma of the myocardium similarly occurred at a significant incidence (P = 0.015) in the low-dose group of males compared with the pooled vehicle controls (controls 0/28, low-dose 6/30, high-dose 2/24) and at a significant incidence (P = 0.002) in the low-dose group of females compared with the pooled vehicle controls (controls 0/28, low-dose 8/27, high-dose 1/12). The survival of both high-dose males and high-dose females was slightly lower than that of the respective low-dose groups and may account for the higher numbers of pulmonary tumors and myocardial sarcomas among low-dose mice of both sexes. The association of myocardial sarcoma with administration of the chemical in both dosed groups of each sex is strengthened by the fact that these tumors of the myocardium have not occurred in the more than 500 male and 500 female historical-control mice of this strain at the laboratory.

Squamous cell carcinoma of the stomach occurred in the dosed male mice (high-dose 2/29) and in the dosed female mice (low-dose 2/26, high-dose 2/14) but was absent in all controls. Although the incidences in this bioassay were too low to be statistically significant, the fact that no squamous-cell carcinomas of the stomach have occurred in the more than 500 male and 500 female historical-control mice of this strain at this laboratory indicates that these gastric tumors were related to the administration of the estradiol mustard.

It is concluded that under the conditions of this bioassay, estradiol mustard administered in a buffered saline vehicle was not carcinogenic in Sprague-Dawley rats. Estradiol mustard was carcinogenic in both male and female B6C3F$_1$ mice, inducing lymphoma, sarcoma of the myocardium, alveolar adenoma or carcinoma, and squamous-cell carcinoma of the stomach.

Synonym: estradiol, bis((p-bis(2-chloroethyl)-amino) phenyl)acetate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-60 Bioassay of Phenesterin for Possible Carcinogenicity (CAS No. 3546-10-9)

Phenesterin, an experimental anticancer agent, is a steroidal alkylating agent composed of the carboxylic acid ester of cholesterol and an aryl nitrogen mustard.

A bioassay of phenesterin for possible carcinogenicity was conducted by administering the chemical by gavage to Sprague-Dawley rats and B6C3F$_1$ mice.

Groups of 35 rats of each sex were administered phenesterin at one of two doses, either 5 or 10 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 32 or 33 weeks. The vehicle used was 0.05% polysorbate 80 in buffered saline. Controls consisted of groups of 10 rats of each sex which received the vehicle (vehicle control) and 10 rats of each sex which were untreated (untreated control). All surviving rats were killed at 84 or 85 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 15 or 30 mg/kg body weight, three times per week for 52 weeks. The males receiving 15 mg/kg were observed for an additional period of 29 weeks, and those surviving to this time were then killed; the animals of the remaining groups were observed for additional periods of only 10-22 weeks, due to early deaths. Seventy-seven weeks after the foregoing groups were started, additional groups of 40 mice of each sex were started and were administered the chemical at 7 mg/kg body weight three times per week; administration of the chemical terminated at week 102 for the males and at week 88 for the females, due to deaths of all females at this time. Controls for the low-dose (7 mg/kg) groups of mice consisted of groups of 20 mice of each sex which received the vehicle (vehicle control) and 20 mice of each sex which were untreated (untreated control); controls for the mid-dose (15 mg/kg) and the high-dose (30 mg/kg) controls consisted of groups of 15 mice of each sex similarly receiving the vehicle or untreated. All surviving low-dose controls were killed at 104 weeks, and all surviving mid- and high-dose controls were killed at 81-84 weeks.

Phenesterin was toxic to rats and mice at the doses used, as shown by reduced mean body weights and survival. Time-adjusted analyses were used for evaluation of incidences of tumors in the female mice.

In female rats, a dose-related trend (P = 0.019) was present in adenocarcinoma of the mammary gland, using the pooled controls, and the incidences of the tumor in the individual dosed groups were significant (P < 0.009) when compared with those in the pooled controls (controls 1/18, low-dose 12/29, high-dose 12/30).

In male mice, the incidence of alveolar/bronchiolar carcinomas or combined alveolar/bronchiolar adenomas and carcinomas in the low-dose group (18/40) was significantly higher (P < 0.020) than that in the low-dose vehicle-control group (0/16). In female mice, seven low-dose animals had alveolar/bronchiolar adenomas and eight other low-dose animals had alveolar/bronchiolar car-

cinomas. When these tumors were combined, their time-adjusted incidence was significant (P = 0.004) when compared with that in the low-dose vehicle controls (controls 1/18, low-dose 15/35). The lower and nonsignificant incidences of these tumors observed in the mid- and high-dose groups may be due to the earlier mortality in these groups compared with the low-dose groups.

In each sex of mid- and high-dose mice, incidences of lymphoma and leukemia were dose related (P<0.005), using vehicle controls; they were also significant (P<0.018) in direct comparisons of mid- and high-dose groups of both sexes with respective vehicle controls (males: controls 0/14, mid-dose 9/29, high-dose 11/25; females, time-adjusted: controls 0/15, mid-dose 14/18, high-dose 17/19). The significance of the incidence of lymphoma and leukemia in the mid- and high-dose groups of males was increased (P<0.001) when the pooled-control group was used, both in the test for dose-related trend and in tests for direct comparisons of dosed groups with the controls.

In each sex of mice, sarcomas of the myocardium were found in all groups of dosed animals, but in no control animals (males: low-dose 5/40, mid-dose 7/29, high-dose 2/25; females: low-dose 8/34, mid-dose 2/7, high-dose 3/7). In males, the incidence in the mid-dose group was significant when compared with that in the pooled controls (P = 0.006); in females, the incidences in the low- and high-dose groups were significant (P<0.023).

It is concluded that under the conditions of this bioassay, phenesterin was carcinogenic in female Sprague-Dawley rats, producing adenocarcinomas of the mammary gland, and in both sexes of $B6C3F_1$ mice, producing alveolar/bronchiolar carcinomas, hematopoietic tumors, and myocardial sarcomas.

Synonym: cholesteryl p-bis(2-chloroethyl)-minophenylacetate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-61 Bioassay of Pentachloronitrobenzene for Possible Carcinogenicity (CAS No. 82-68-8)

Pentachloronitrobenzene (PCNB), a halogenated benzene derivative and agricultural pesticide, was selected for bioassay by the National Cancer Institute following its classification as a tumorigenic agent by the Secretary's Commission on Pesticides and Their Relationship to Environmental Health.

A bioassay of technical-grade pentachloronitrobenzene (PCNB) for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. PCNB was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average dietary concentrations of PCNB were, respectively, 10,064 and 5,417 ppm for male rats, 14,635 and 7,875 ppm for female rats, 5,213 and 2,606 ppm for male mice, and 8,187 and 4,093 ppm for female mice. After a 78-week period of compound administration, observation of the rats continued for an additional 33 to 35 weeks and observation of the mice continued for 14 or 15 additional weeks.

For each species, 20 animals of each sex were placed on test as controls and fed only the basal diet.

No rare or unusual tumors were observed during the histopathologic examinations and no statistically significant positive associations were demonstrated between chemical administration and the incidence of neoplasms in either sex of either species.

It is concluded that under the conditions of this bioassay PCNB was not carcinogenic in either Osborne-Mendel rats or $B6C3F_1$ mice.

Synonyms: quintozene; terrachlor; PCNB

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

Note: Pentachloronitrobenzene was subsequently studied by administration in feed to $B6C3F_1$ mice (See TR-325, reported 1978).

TR-62 Bioassay of Endosulfan for Possible Carcinogenicity (CAS No. 115-29-7)

Endosulfan is a synthetic chlorinated cyclodiene and was introduced in 1956 as a broad spectrum insecticide.

A bioassay of technical-grade endosulfan for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Endosulfan was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species.

The time-weighted average high and low dietary concentrations of endosulfan were, respectively, 952 and 408 ppm for the male rats, and 445 and 223 ppm for the female rats. In mice the high and low time-weighted average concentrations were, respectively, 6.9 and 3.5 ppm for the males and 3.9 and 2.0 ppm for the females. Twenty animals of each sex and species were placed on test as controls. The bioassay of high dose male rats was terminated during week 82, and the bioassay of low dose male rats was terminated during week 74. After a 78-week period of chemical administration, observation of female rats continued for 33 additional weeks and observation of mice continued for 14 additional weeks.

At the doses administered to rats in this study endosulfan was toxic, inducing a high incidence of toxic nephropathy in both sexes and testicular atrophy in males.

In both species high early mortality was observed in the male groups and no conclusions concerning the carcinogenicity of endosulfan can be drawn from this part of the bioassay. However, survival among females of both species was sufficient for meaningful statistical evaluation of the incidence of late-developing tumors. It is concluded that under the conditions of this bioassay, technical-grade endosulfan was not carcinogenic in female Osborne-Mendel rats or in female B6C3F$_1$ mice.

Synonym: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Negative
Male Mice:	Inadequate Study
Female Mice:	Negative

TR-63 Bioassay of 4-Chloro-o-phenylenediamine for Possible Carcinogenicity (CAS No. 95-83-0)

4-Chloro-o-phenylenediamine, an aromatic amine used as an intermediate in dye production, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among dye manufacturing industry workers.

A bioassay for the possible carcinogenicity of technical-grade 4-chloro-o-phenylenediamine was conducted using Fischer 344 rats and B6C3F$_1$ mice. 4-Chloro-o-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. For male and female rats, the high and low time-weighted average dietary concentrations of 4-chloro-o-phenylenediamine were 1.0 and 0.5 percent, respectively. For male and female mice, the high and low time-weighted average dietary concentrations were 1.4 and 0.7 percent, respectively. After a 78-week period of chemical administration, observation of the rats continued for up to an additional 28 weeks and observation of the mice continued for up to an additional 18 weeks. Fifty animals of each species and sex were placed on test as controls for the chronic bioassay.

There was a statistically significant positive association between increased dosage and accelerated mortality in female rats and male mice; however, survival among all groups of was adequate for meaningful statistical analysis of late-developing tumors.

In male and female rats receiving the test chemical, a significantly increased incidence of neoplasms of the urinary bladder occurred. Neoplastic nodules in the liver and tumors of the forestomach may also have been related to administration of the chemical. A significantly increased incidence of hepatocellular carcinomas occurred in chemically treated male and female mice.

It is concluded that under the conditions of this bioassay 4-chloro-o-phenylenediamine was carcinogenic in Fischer 344 rats and B6C3F$_1$ mice, including tumors of the urinary bladder and forestomach in both sexes of rats and hepatocellular carcinomas in both sexes of mice.

Synonyms: 4-chloro-1,2-benzenediamine; 4-chloro-1,2-diaminobenzene; Ursol Olive 6G

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-64 Bioassay of 1-Nitronaphthalene for Possible Carcinogenicity (CAS No. 86-57-7)

1-Nitronaphthalene is used as an intermediate for the preparation of 1-naphthylamine, which is used in the manufacture of numerous dyes and intermediates, and in the production of rodenticides. 1-Nitronaphthalene is also sulfonated to produce 1-nitronaphthalene-5-sulfonic acid, a dye intermediate. 1,5- and 1,8-Dinitronaphthalenes, produced by further nitration of 1-nitronaphthalene, have had limited use in the dye industry. 1-Nitronaphthalene is also used as a deblooming agent for petroleum and oils (in concentrations of 2-3 parts/1,000 parts oil), and as a modifier to decrease the burning rate of explosives.

A bioassay of technical-grade 1-nitronaphthalene for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. 1-Nitronaphthalene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low time-weighted average concentrations used in the chronic study were, respectively, 0.18 and 0.06 percent for rats and 0.12 and 0.06 percent for mice. After a 78-week period of chemical administration, the rats were observed for an additional period of up to 31 weeks and the mice for an additional period of up to 20 weeks. For rats 50 animals of each sex were placed on test as controls for the low dose groups and 25 of each sex for the high dose groups. For mice 50 animals of each sex were placed on test as controls for each dosed group.

In both species adequate numbers of animals in all groups survived sufficiently long for the development of late-appearing tumors; however, no compound-related increase in the incidence of neoplasms, nonneoplastic lesions, or other toxic effects was evident.

Under the conditions of this bioassay 1-nitronaphthalene was not demonstrated to be carcinogenic in Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: alpha-nitronaphthalene, nitronaphthalene

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-65 Bioassay of Chloropicrin for Possible Carcinogenicity (CAS No. 76-06-2)

Chloropicrin is an agricultural fumigant, once widely used but now being phased out. It was developed as a tear gas, but was found to be useful as a fumigant in 1918. The primary use of chloropicrin as a fumigant was in the treatment of stored grain. It also functions as a nematicide, fungicide, and insecticide when used as a soil fumigant prior to planting.

A bioassay of technical-grade chloropicrin for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Chloropicrin in corn oil was administered 5 days a week by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. Time-weighted average dosages of 25 mg/kg/day for low dose male rats and 20 mg/kg/day for low dose female rats were administered during weeks 1 through 33, then administered cyclically (1 dose-free week followed by 4 weeks of administration) from weeks 34 through 78. Time-weighted average dosages of 26 mg/kg/day for high dose male rats and 22 mg/kg/day for high dose female rats were administered from weeks 1 through 17, weeks 31 through 33, and cyclically (1 dose-free week followed by 4 weeks of administration) during weeks 34 through 78. Time-weighted average dosages of 66 and 33 mg/kg/day, respectively, for male and female mice were administered for 78 weeks. These dosing regimens were followed by observation periods of 32 weeks for rats and 13 weeks for mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not gavaged.

A high incidence of early death was observed among chloropicrin-dosed rats. Deaths among dosed rats occurred as early as week 1 for females and week 6 for males. Median survival was week 48 for high dose males, week 54 for low dose males, week 70 for high dose females and week 59 for low dose females. Statistical tests indicate a positive association between chloropicrin dosage and mortality of rats.

No neoplasms were observed at higher incidences in dosed than control rats. In rats of both sexes, incidences of adenoma of the pituitary and of adenocarcinoma or fibroadenoma of the mammary gland were higher in control groups than dosed groups. It is likely that most dosed rats did not survive long enough to be at risk from late-appearing tumors.

A rapid decrease in survival after the first year of the study was observed among high dose mice of both sexes. Survival of high dose male mice decreased from 80 percent in week 54 to 26 percent in week 90. Survival of high dose female mice decreased from 82 percent in week 54 to 36 percent in week 90. Statistical tests indicated a positive association between chloropicrin dosage and mortality of mice.

In chloropicrin-dosed mice, proliferative lesions of the squamous epithelium of the forestomach included two carcinomas and a papilloma. Although these tumors were uncommon in control animals, statistical analysis did not demonstrate that they were related to administration of chloropicrin. Other proliferative lesions of the forestomach occurring at an increased incidence in dosed mice were acanthosis and hyperkeratosis. No statistically significant increase of tumor incidence was observed in mice.

The bioassay of chloropicrin using Osborne-Mendel rats did not permit an evaluation of carcinogenicity because of the short survival time of dosed animals. The bioassay of chloropicrin using B6C3F$_1$ mice did not provide conclusive statistical evidence for the carcinogenicity of this compound.

Synonyms: trichloronitromethane, nitrochloroform

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Negative
Female Mice:	Negative

TR-66 Bioassay of 1,1-Dichloroethane for Possible Carcinogenicity (CAS No. 75-34-3)

1,1-Dichloroethane is used as a chemical intermediate and as a solvent for extraction and degreasing.

A bioassay of technical-grade 1,1-dichloroethane for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. 1,1-Dichloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, 5 days a week for a period of 78 weeks, followed by an observation period of 33 weeks for rats and 13 weeks for mice.

A preliminary subchronic toxicity test, consisting of 6 weeks of 1,1-dichloroethane administration at five dosage levels followed by 2 weeks of observation, was performed for the purpose of selecting initial dosages. Subsequent dosage adjustments were made during the course of the study. The high and low time-weighted average dosages of 1,1-dichloroethane were, respectively, 764 and 382 mg/kg/day for male rats; 950 and 475 mg/kg/day for female rats; 2,885 and 1,442 mg/kg/day for male mice; and 3,331 and 1,665 mg/kg/day for female mice.